Mechanical impact on the head has an antihypertensive effect

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Abstract

Nervous cell functions are known to be physiologically regulated by mechanical factors in the brain. However, it remains unclear whether mechanical interventions can modulate the pathophysiological processes underlying brain-related disorders and modify their consequences. Here we show that passive head motion of hypertensive rats, which reproduces mechanical accelerations generated at their heads during treadmill running at a moderate velocity, decreases the expression of angiotensin II type 1 receptor (AT1R) in astrocytes in their rostral ventrolateral medulla (RVLM). This decrease results in lowering their blood

pressure. Passive head motion generates interstitial fluid movement that is estimated to exert shear stress with average magnitude of a few Pa on cells in rats' brainstem. Fluid shear stress of a relevant magnitude decreases AT1R expression in cultured astrocytes, but not in neuronal cells. Furthermore, in hypertensive rats, inhibition of movement of interstitial fluid by its gelation with reactive polyethylene glycol injected into the RVLM eliminates the ability of passive head motion to decrease their blood pressure and AT1R expression in RVLM astrocytes. Consistent with these results from animal experiments, vertically oscillating chair riding of hypertensive adult humans, which reproduces mechanical accelerations generated at their heads during light jogging or fast walking, lowers their blood pressure. Our findings indicate that moderate mechanical impact on the head has an antihypertensive effect by modulating the function of RVLM astrocytes through interstitial fluid shear stress. We anticipate mechanical regulation to underlie a variety of positive effects of physical exercise on human health, particularly those related to brain functions.

Introduction

Hypertension, which is a major cause of stroke and cardiovascular diseases, is the biggest risk factor for death worldwide¹. Whereas numerous antihypertensive drugs have been developed and used clinically, physical exercise is known to be effective for the treatment and prevention of essential hypertension^{2,3}, which comprises the majority (>90%) of human hypertension⁴. Although

long-term regulation of blood pressure (BP) has been recognized to be largely dependent on sodium excretion adjusting systems, which mainly involve kidney functions⁵, elevated activity of the sympathetic nervous system also importantly contributes to the development of hypertension⁶⁻⁸. Rostral ventrolateral medulla (RVLM), which is located in the brainstem, plays a critical role in determining the basal activity of the sympathetic nervous system, and its functional integrity is essential for the maintenance of basal vasomotor tone and regulation of BP^{6,9}. Angiotensin II (Ang II) is the major bioactive peptide of the renin-angiotensin system (RAS), and is known to regulate BP as well as other biological processes such as cell growth/apoptosis/migration, inflammation, and fibrosis¹⁰. The biological effects of Ang II are mediated by its interaction with two distinct high-affinity G protein-coupled receptors, Ang II type 1 receptor (AT1R) and type 2 receptor. Of these receptors, AT1R is responsible for most of the known physiological and pathophysiological processes related to Ang II. Whereas RAS is involved in the functional regulation of various “peripheral” organs and tissues such as kidney and vessels, it also regulates brain functions within the blood-brain barrier, including the control and maintenance of sympathetic nerve activity and cognitive ability¹¹. In particular, the role of AT1R signaling in the RVLM in cardiovascular regulation has been extensively studied and demonstrated. For example, the pressor/depressor responses to Ang II and Ang II antagonists, injected into the RVLM have been reported to be enhanced in spontaneously hypertensive rats (SHRs)^{12,13}. We have previously demonstrated that

treadmill running at moderate velocities alleviates the sympathetic nerve activity, involving attenuation of AT1R signaling in the RVLM of stroke-prone spontaneously hypertensive rats (SHRSPs)¹⁴, a substrain of SHRs that exhibit severer hypertension as compared with SHRs¹⁵. However, the details about the changes in AT1R signaling in the RVLM of these hypertensive rats have yet to be elucidated. It remains unclear what type(s) of cells (e.g., neurons or astrocytes) are primarily responsible for the altered AT1R signaling in the RVLM of SHRs or SHRSPs. Furthermore, the causal relationship between the increased AT1R signal activity in the RVLM and high BP in SHRs or SHRSPs in their steady state (i.e., apart from their responses to pharmacological interventions) is left unrevealed.

AT1R has also been shown to play a vital role in regulating a variety of physiological or pathological processes, including cellular responses to mechanical perturbations^{16,17}. For example, mechanical stretching of cardiac myocytes activates AT1R signaling¹⁸, and fluid shear stress (FSS) of average 1.5 Pa lowers AT1R expression in human vein endothelial cells¹⁹. Although intervening the Ang II-AT1R system through pharmacological approaches, such as administration of angiotensin-converting enzyme inhibitor or selective AT1R blocker, has been established as an effective therapeutic strategy for hypertension²⁰, mechano-responsive attenuation of AT1R signaling has not been clinically utilized as an antihypertensive measure.

Many of physical workouts, particularly aerobic exercises, involve vertical body motions,

which generate mechanical impacts (accelerations) on the head at the time of foot contact with the ground (i.e., landing). The importance of mechanical loads is well established in the physiological regulation of bones, the stiffest organ that only allows tiny deformation²¹. Osteocytes, the mechanosensory cells embedded in bones²², are assumed to undergo minimal deformations under physiological conditions. We have reported that FSS on osteocytes derived from interstitial fluid flow induced upon physical activity plays an important role in maintaining bone homeostasis²³. Given that the brain is not a rigid organ, minimally deforming forces or stress distribution changes in the brain during exercise or even activity of daily living (e.g., walking) may produce beneficial effects. We have previously shown that in the prefrontal cortex (PFC) of rodents, moderate mechanical impact-induced FSS modulates serotonin signaling in the neurons in situ²⁴. Based on these previous findings together with the distribution of interstitial fluid throughout the whole brain, we hypothesized that moderate mechanical impact on the head might have antihypertensive effects involving FSS-mediated modulation of AT1R signaling in the RVLM.

Results

Application of cyclical mechanical impact to the head by passive motion lowers the BP in

SHRSPs. To determine the effects of a mechanical intervention of a moderate intensity on BP, we first sought to develop an experimental system that reproduces the impact exerted on the head

111 during rats' treadmill running at a modest velocity (20 m/minute), a typical experimental
112 intervention to test the effects of physical exercise on rats^{25,26}. In a recent study, we observed that
113 treadmill running of rats (20 m/minute) generated 5-mm vertical oscillation of their heads with ~1.0
114 x g peak accelerations and 2-Hz frequency; therefore, we developed a "passive head motion"
115 (PHM) system to produce 2-Hz 5-mm vertical oscillation exerting 1.0 x g acceleration peaks at the
116 heads of rats²⁴. In the current study, we examined the effects of mechanical impact on BP in
117 SHRSPs, using the PHM system. Similar to the antihypertensive effect of treadmill running on
118 SHRs or SHRSPs that we and others reported previously^{14,25,27}, application of PHM (30
119 minutes/day, 28 consecutive days; see Fig. 1a) significantly lowered their BP (Fig. 1b,c) as
120 compared to their controls, whereas HR was not significantly affected by PHM (Fig. 1d).
121 Anesthesia alone (daily 30 minutes) did not significantly alter the BP in SHRSPs (Extended Data
122 Fig. 1a), indicating that the antihypertensive effect resulted specifically from PHM. The anti-cardiac
123 hypertrophy effect of PHM on SHRSPs (Fig. 1e) as well as the lack of these PHM effects on control
124 normotensive rats (Wistar-Kyoto: WKY) (Fig. 1b,c,e) were also consistent with previous reports
125 describing treadmill running as an antihypertensive intervention for SHRs²⁷. As was observed in our
126 treadmill running experiments¹⁴, PHM decreased 24-hour urinary norepinephrine excretion of
127 SHRSPs (Fig. 1f). This suggests that PHM mitigates the sympathetic hyperactivity²⁸. Collectively,
128 these results support our hypothesis that cyclical moderate mechanical impact on the head has an

antihypertensive effect.

PHM down-regulates AT1R expression in RVLM astrocytes of SHRSPs. We then looked into the mechanism of how PHM alleviated the development of hypertension in SHRSPs. We previously reported that down-regulation of AT1R signaling in the RVLM is responsible for the treadmill running-induced sympathoinhibition in SHRSPs¹⁴. Given the mechanical regulation of AT1R expression in endothelial cells¹⁹, we examined whether PHM modulated AT1R expression in RVLM neurons and astrocytes of SHRSPs. In our histochemical analysis, we defined neuronal nuclei (NeuN)-positive cells as neurons²⁹ and glial fibrillary acidic protein (GFAP)-positive cells as astrocytes³⁰. PHM (30 minutes/day, 28 days) did not significantly change the relative population of AT1R-expressing neurons and astrocytes in the RVLM of WKY rats (Fig. 1g). In contrast, 4-week PHM significantly decreased the expression of AT1R in the astrocytes, but not in the neurons, of SHRSPs' RVLM (Fig. 1h). Notably, AT1R expression in the RVLM neurons was comparable between WKY rats and SHRSPs, either with and without PHM (Fig. 1i). In contrast, AT1R expression was significantly higher in the RVLM astrocytes of SHRSPs without PHM (Fig. 1j, column 3). PHM lowered the AT1R expression in the RVLM astrocytes of SHRSPs to the level equivalent to that of WKY rats (Fig. 1j, columns 1, 2, and 4). Taken together, AT1R expression in RVLM astrocytes appeared to be correlated with the antihypertensive effect of PHM on SHRSPs. In

line with this observation, 4-week treadmill running of SHRSPs also decreased the AT1R expression in their RVLM astrocytes, but not neurons (Extended Data Fig. 1b–d).

PHM alleviates the sensitivity of RVLM in SHRSPs to Ang II or Ang II antagonist. We next sought to examine whether the PHM-induced decrease in AT1R expression in the RVLM astrocytes of SHRSPs (Fig. 1j, columns 3 and 4) was functionally relevant to the suppression of AT1R signaling. To this end, we analyzed the pressor responses to Ang II injected into unilateral RVLM of WKY rats and SHRSPs, either subjected to 4-week PHM or left sedentary under anesthesia (30 minutes/day, 28 days) (Fig. 2a). As we previously reported¹⁴, SHRSPs without PHM exhibited significantly greater pressor response to Ang II administered to RVLM than WKY rats (Fig. 2b, compare between top and bottom of left panels; Fig. 2c, compare columns 1 and 3). Four-week PHM ameliorated the pressor response to Ang II injected into the RVLM of SHRSPs, but not of WKY rats (Fig. 2b, compare left and right; Fig. 2c, compare columns 1 vs. 2 and 3 vs. 4). Furthermore, depressor responses to Ang II antagonist injected into unilateral RVLM¹³ of WKY rats and SHRSPs with and without 4-week PHM appeared to be approximate mirror images of the pressor responses (Fig. 2d,e). These results support the functional relevance of the PHM-induced decrease in AT1R expression in the RVLM astrocytes of SHRSPs (Fig. 1j).

To examine whether the increased AT1R expression in the RVLM astrocytes of SHRSPs was

associated with their development of hypertension, we manipulated AT1R signaling by introducing exogenous expression of AT1R-associated protein (AGTRAP), which interacts with AT1R and tempers Ang II-mediated signals by promoting AT1R internalization³¹. To this end, we used an adeno-associated virus (AAV)-mediated gene delivery system³². AAV serotype 9 (AAV9) vectors were injected locally to transduce RVLM cells (Fig. 3a and Extended Data Fig. 2a). To achieve astrocyte- and neuron-specific gene expression, we used AAV9 vectors that harbored mouse GFAP promoter (AAV-GFAP) and rat neuron-specific enolase (NSE) promoter (AAV-NSE), respectively (Fig. 3a). Because these vectors contained a region encoding GFP and 2A sequence of porcine teschovirus-1 (P2A; self-cleaving peptides³³) (Fig. 3a), observation of green fluorescence allowed us to identify the cells in which transgene was expressed (Fig. 3b,c and Extended Data Fig. 2a–e). AAV-mediated expression of AGTRAP in astrocytes (Fig. 3b) but not in neurons (Fig. 3c) of bilateral RVLMs in SHRSPs significantly lowered the BP as compared with their control SHRSPs in which only GFP was virally expressed in RVLM astrocytes or neurons (Fig. 3d,e). Furthermore, AAV-mediated expression of AGTRAP in astrocytes, but not neurons, of SHRSPs' bilateral RVLMs decreased 24-hour urinary norepinephrine excretion (Fig. 3f). Injection of control AAV vector (GFAP-control or NSE-control) did not significantly affect the BP of SHRSPs (Extended Data Fig. 2f). These results support the importance of AT1R signal intensity in RVLM astrocytes for SHRSPs' development of hypertension and sympathetic hyperactivity, as well as the physiological relevance

of PHM-induced decrease in AT1R expression we observed in the RVLM astrocytes of SHRSPs (Fig. 1j).

PHM generates low-amplitude pressure waves and induces interstitial fluid movement in rat

RVLM. We then sought to determine the physical effects that PHM produced in rat RVLM. To do

so, we analyzed local pressure changes using a telemetry pressure sensor (Fig. 4a) as we described

previously²⁴. PHM generated pressure waves (changes) with ~1.2 mm Hg peak amplitude (Fig.

4b–d). Hydrostatic pressure of this magnitude (~1.6 cm H₂O) is unlikely to initiate mechano-

responsive signaling in cells³⁴. Postulating an analogy to osteocytes embedded in bones, the

function of which is known to be modulated by interstitial fluid flow-derived shear stress²³, we have

demonstrated that minimal stress distribution changes generate interstitial fluid flow in the brain,

resulting in shear stress-mediated regulation of nervous cell functions²⁴.

To analyze the PHM-induced interstitial fluid movement in the RVLM, we injected an iodine-

based contrast agent (Isovist[®]) into the RVLM of anesthetized rats, and tracked its distribution with

sequential computed tomography (CT) (Fig. 4e) as we previously did to quantify the movement of

intramuscular interstitial fluid³⁵. We found that PHM significantly promoted Isovist spreading in the

rostral-caudal and dorsal-ventral (y- and z-axes, Fig. 4f) directions (Fig. 4g,h). In contrast, PHM did

not significantly affect the left-right spreading (x-axis, Fig. 4f) of Isovist (Fig. 4g,h). As we

observed in rat PFC²⁴, PHM induced interstitial fluid movement in the brainstem in defined directions, rather than in an isotropic manner. Our simulative calculation suggests that PHM subjected RVLM cells to interstitial fluid flow-derived shear stress with an average magnitude of 0.59–2.64 Pa (Supplementary Table 1). FSS of this magnitude is known to modify the physiological function of astrocytes³⁶, leading us to hypothesize that FSS derived from interstitial fluid movement (Fig. 4e–h) mediated the PHM-induced decrease in AT1R expression in RVLM astrocytes (Fig. 1j).

FSS on astrocytes decreases AT1R expression in vitro. To test this hypothesis, we conducted in vitro FSS experiments. Based on our simulation (Supplementary Table 1), we applied pulsatile FSS with an average magnitude of 0.7 Pa to cultured primary astrocytes, which were prepared from astrocyte-GFP mice³⁷ (Extended Data Fig. 3a), using a system we previously reported^{23,24,35,38}. Quantitative polymerase chain reaction (qPCR) and immunostaining analyses revealed that FSS application (0.5 Hz, 30 minutes) significantly decreased AT1R expression in astrocytes for at least 24 hours (Fig. 5a–c). In contrast, FSS application to Neuro2A cells, which exhibit neuronal phenotypes and morphology^{39,40}, did not decrease AT1R expression (Extended Data Fig. 3b–d).

Consistent with the decreasing effect of FSS on AT1R expression, the binding of fluorescently labeled Ang II to cultured astrocytes was significantly decreased by pre-exposure to 30-minute FSS (Fig. 5d,e). The duration (>24 hours) of FSS effects on AT1R expression in astrocytes (Fig. 5) poses

a possibility of cumulative effects of FSS applied repeatedly at 24-hour intervals. Therefore, these in vitro observations suggest that FSS-mediated persistent reduction of AT1R expression is involved in the effects of daily PHM application on BP (Fig. 1b,c) and AT1R expression in the RVLM astrocytes (Fig. 1j) in SHRSPs.

Hindrance of interstitial fluid movement by hydrogel introduction in RVLM eliminates the ability of PHM to decrease the AT1R expression in RVLM astrocytes and the BP in SHRSPs.

To examine whether interstitial fluid movement in the RVLM mediated the effects of PHM on BP and AT1R expression in RVLM astrocytes of SHRSPs, we modulated local interstitial fluid dynamics. Following the procedure we used to hinder the interstitial fluid movement in mouse PFC²⁴, we gelled interstitial fluid in situ and deprived its fluidity by microinjecting mutually reactive polyethylene glycol (PEG) gel-precursor (pre-gel) solutions to RVLM (Fig. 6a). Injected pre-gel solution spread over rat RVLM, and gelled the interstitial fluid in situ (Extended Data Fig. 4a).

Hydrogel introduction in bilateral RVLMs eliminated the ability of PHM to decrease BP (Fig. 6b, black and orange lines; Fig. 6c, columns 2 and 3), urinary norepinephrine excretion (Fig. 6d, columns 2 and 3), and AT1R expression in the RVLM astrocytes (Fig. 6e, bottom 2 rows; Fig. 6f, columns 2 and 3) in SHRSPs. In contrast, hydrogel introduction increased BP (Fig. 6b, blue and

orange lines; Fig. 6c, columns 1 and 3), norepinephrine excretion (Fig. 6d, columns 1 and 3), and AT1R expression in the RVLM astrocytes (Fig. 6e, top 2 rows; Fig. 6f, columns 1 and 3) of SHRSPs subjected to PHM. AT1R expression in the RVLM neurons of SHRSPs remained unaltered irrespective of the combination of PHM and hydrogel introduction (Fig. 6e,g). These results suggest that hydrogel introduction in the RVLM disrupts the mechanism mediating the PHM-induced decrease in BP, norepinephrine excretion, and AT1R expression in the RVLM astrocytes of SHRSPs.

We previously showed that gelation only inhibits the fluidity of the fluid but does not restrict the diffusion of small molecules inside the gel^{24,41}. Consistently, hydrogel introduction did not apparently delay or attenuate the depressor response to Ang II antagonist injected to the RVLM (Extended Data Fig. 4b–d), indicating rapid solute diffusivity through the hydrogels. As was the case with mouse PFC²⁴, gelation by injecting PEG solution did not affect overall cell number or apoptosis in RVLM (Extended Data Fig. 4e,f), and survival or apoptosis of RVLM astrocytes (Extended Data Fig. 4g,h) and neurons (Extended Data Fig. 4i,j). Collectively, the loss of PHM effects by hydrogel introduction in bilateral RVLMs of SHRSPs (Fig. 6b–f) is likely to result from hydrogel-mediated alteration in interstitial fluid dynamics, rather than decreased cell viability caused by impaired nutrient supply or removal of metabolic wastes.

Vertically oscillating chair riding (VOCR) lowers BP in hypertensive adult humans. The

results from our animal experiments reveal the antihypertensive effect of mechanical accelerations generated at the head during treadmill running at a moderate velocity. This prompted us to test whether mechanical impact on the head lowered BP in hypertensive humans. As we observed light jogging or fast walking (locomotion at the velocity of 7 km/hour) typically produce ~2 Hz vertical acceleration waves with an amplitude of ~1.0 x g at the person's head (Extended Data Fig. 5a, top), we constructed a chair that could vertically oscillate at the frequency of 2 Hz (Extended Data Fig. 5b) and produce ~1.0 x g acceleration waves at the head of the occupant (Extended Data Fig. 5a, bottom).

Given that previous reports regarding antihypertensive effects of aerobic exercise typically recommend ≥ 3 –4 days per week (frequency) and ≥ 30 minutes per session or day (duration)³, we set our regimen of VOCR as 3 days/week (Monday, Wednesday, and Friday unless needed to assign otherwise for particular reasons such as public holidays) and 30 minutes/day. Our study of protocol 1, in which we simply compared the subjects' BP and HR before and after 4-week (12 times) VOCR (Extended Data Fig. 5c), showed that VOCR decreased BP in hypertensive humans (Fig. 7a).

We then conducted a human study of protocol 2, in which we followed the changes in subjects' BP and HR more minutely (Extended Data Fig. 5d). Encouraged by the positive results from the

study of protocol 1, we adopted the same VOQR regimen as to its frequency (3 days/week) and duration (30 minutes/day). Participants were subjected to serial blood sampling to measure plasma catecholamines (epinephrine, norepinephrine, and dopamine) and renin activity, and serum aldosterone and C-reactive protein (CRP) before and after the intervention period (Extended Data Fig. 5d). To conduct the 2nd blood sampling on the next day of the last bout of VOQR, the intervention period was extended from 4 weeks (total 12 times, typically 26 days) to 4.5 weeks (total 14 times, 30–31 days) because blood sampling could not be done during weekends at our hospital. BP, both systolic and diastolic, and MAP immediately after the intervention period significantly decreased as compared with those immediately before the intervention period (Fig. 7b). Furthermore, we also observed an antihypertensive effect of VOQR when we defined “BP of the week” to reliably detect the trends by reducing the influences from interday BP variability (see Methods) (Fig. 7c and Extended Data Fig. 6a). Notably, the post-intervention follow-up showed that the BP-lowering effect apparently persisted for 4 weeks, but not 5 weeks, after the last bout of VOQR (Fig. 7c). Similar to our animal study, we did not observe significant changes in HR by the VOQR intervention (Fig. 7a–c). Significant differences were not detected in the blood levels of catecholamines, aldosterone, renin activity, and CRP between before and after the VOQR intervention (Extended Data Fig. 6b).

Collectively, our studies of protocols 1 and 2 suggest that VOQR, which reproduces

mechanical impact exerted on the head during light jogging or fast walking, has an antihypertensive effect in hypertensive humans. Importantly, in none of 21 subjects (Supplementary Table 2), apparent adverse events, including motion sickness and low back pain, were observed or manifested in relation to the VOCR intervention.

Discussion

Essential hypertension is defined as high BP in which secondary causes including renovascular disease, renal failure, pheochromocytoma, and primary aldosteronism are not present. It accounts for >90% of human cases of hypertension, resulting from combinations of multiple genetic and environmental factors⁴. Recently, brain (dys)function has been implicated in the pathogenesis of essential hypertension; however, the details of their molecular link remain unclear⁴². Physical exercise is proven to be effective as a therapeutic/preventative measure for essential hypertension³. Although the antihypertensive effect of physical exercise has been shown to involve normalization of sympathetic hyperactivity in the brain⁴³, it is still unclear whether exercise directly modulates brain function. In this study, PHM, which reproduced mechanical accelerations generated at the head during treadmill running, allowed us to dissect bodily activity-derived physical effects.

Whereas AT1R signaling in both neurons and astrocytes of the RVLM have been reported to be involved in regulating BP^{44,45}, we observed that AT1R expression in RVLM astrocytes was

increased in SHRSPs as compared to that in WKY rats (Fig. 1j). In contrast, AT1R expression in RVLM neurons was comparable between WKY rats and SHRSPs (Fig. 1i), although AT1R expression in RVLM neurons has been shown to play an important role in other animal model(s) of hypertension⁴⁴. Together with the decreases in BP and urinary epinephrine excretion of SHRSPs in which RVLM astrocytes were transduced with AGTRAP gene (Fig. 3d–f), the intensity of AT1R signaling in RVLM astrocytes appears to be critically involved in the pathogenesis of hypertension and sympathetic hyperactivity in SHRSPs.

Four-week PHM decreased urinary norepinephrine excretion and AT1R expression in RVLM astrocytes of SHRSPs to the levels almost equivalent to those of WKY rats (Fig. 1f,j). However, PHM only partially alleviated the development of hypertension in SHRSPs to the extent similar to the antihypertensive effects of treadmill running we and others previously reported^{14,25} (Fig. 1b,c). Therefore, it is evident that factors other than AT1R signaling in RVLM astrocytes contribute to the pathogenesis of essential hypertension.

AT1R expression in cultured astrocytes decreased upon FSS application (Fig. 5a–c). This was consistent with our findings that PHM and treadmill running decreased in AT1R expression in RVLM astrocytes of SHRSPs (Fig. 1j and Extended Data Fig. 1d). However, the AT1R expression level in RVLM astrocytes was low in WKY rats even without PHM (Fig. 1j), and this may raise a concern regarding the physiological relevance of our in vitro FSS experiments using cultured

astrocytes. Yet, it has been reported that cultured astrocytes typically exhibit increased “reactivity”, and do not fully recapitulate physiological astrocytes *in vivo*⁴⁶. We suggest that the FSS-induced decrease in AT1R expression in cultured astrocytes we observed represents physiological functions of astrocytes, despite that their increased basal AT1R expression may relate to unphysiological aspects of two-dimensional culture on stiff substrates (culture plastics). Cells in static culture are exposed to a complete absence of FSS, which may not be physiologically realized *in vivo*. Previous reports describe increased extracellular fluid in brains of hypertensive humans⁴⁷ and altered dynamics of intracerebral interstitial fluid of SHR⁴⁸. Aberrant regulation of RVLM astrocytes’ function that relates to altered interstitial fluid movement-derived FSS may underlie the pathogenesis of essential hypertension.

PHM did not significantly alter the AT1R expression in SHRSPs’ RVLM neurons (Fig. 1i), and FSS did not decrease AT1R expression in cultured Neuro2A cells (Extended Data Fig. 3b–d). Yet, we do not suspect that these results represent the absence of sensitivity of neurons to FSS or other type(s) of mechanical stimulation, particularly because we observed PHM- and FSS-induced internalization of 5-HT_{2A} receptor expressed in mouse PFC neurons and Neuro2A cells, respectively²⁴. Alternatively, we speculate that PHM and FSS may mitigate hyperexpression of AT1R related to aforementioned pathological status of SHRSPs’ RVLM astrocytes or unphysiological nature of cultured astrocytes. In line with this notion, PHM did not significantly

affect the AT1R expression in RVLM astrocytes in normotensive WKY rats (Fig. 1j). Relevantly, we and others have reported mechanical loading (in vivo)- or FSS (in vitro)- induced alleviation of inflammatory processes^{23,49}, which can transcriptionally increase AT1R expression⁵⁰.

Whereas the amplitude of pressure waves that PHM generated in rat RVLM was tiny (Fig. 4b–d), the magnitude of interstitial fluid movement-derived shear stress in rat RVLM appeared comparable with that of FSS previously reported for vascular endothelium^{51,52}, interstitium of bone²¹ and skeletal muscle³⁵ (Supplementary Table 1b). Consistent with the lack of strict cell specificity in many of cellular responses to mechanical forces⁵³, FSS-induced decrease in AT1R expression, which was reported in vascular cells¹⁹, was also observed in cultured astrocytes. Taken together, we speculate that there may be common homeostasis-regulatory mechanisms at the cellular level that involve fluid flow-derived shear stress of ~0.5 to a few Pa.

Based on our hypothesis concerning the similarity in the pathogenesis of high BP between human essential hypertension and SHRSPs, we conducted human studies in which we intended to reproduce the mechanical impact on the head that lowered the BP in SHRSPs. Although the mechanism behind the apparent antihypertensive effect of VOCR remains to be determined, the significant role of interstitial fluid dynamics in the RVLM, which we demonstrated by our animal experiments, might be shared between humans and rats or other animals (Extended Data Fig. 7). Whereas plasma catecholamine levels were not significantly changed by the VOCR intervention

(Extended Data Fig. 6b), it is possible that urinary epinephrine measures collected over 24 hours in our rat PHM experiments (Fig. 1f, 3f, and 6d) enhanced our ability to capture the sympathetic nerve activity under “everyday-life” ambulatory conditions⁵⁴.

As the phrase “Exercise is Medicine” indicates, physical exercise is broadly useful to maintain human health. Many of aerobic exercises, including walking and running, involve impact-generating bodily actions creating sharp accelerations at the head upon foot contacting with the ground. Therefore, their beneficial effects as therapeutic/preventative procedures for a variety of brain function-related diseases and health disorders may rely at least partly on modest changes in mechanical stress distribution in the brain, which may prompt optimal FSS on intracerebral nervous cells. While we have recently demonstrated that brain function can be physiologically regulated by mechanical forces²⁴, alterations in interstitial fluid movement-derived shear stress may underlie the pathogenesis of various brain disorders, particularly those related to physical inactivity or aging.

Limitation of study. There are several limitations of this study. We were unable to test the response of primary neurons, which were prepared from mouse cerebral cortex or hippocampus, to FSS of relevant magnitudes (0.59–2.64 Pa, see Supplementary Table 1b) because of their easy detachment from the substrates by FSS. As an alternative of cultured neuronal cells, we tested Neuro2A cells, which stably adhered to the substrates through FSS of magnitudes up to ~1 Pa²⁴.

We did not comprehensively analyze the effects of PHM on brain functions, but focused on the

study of RVLM. PHM may modulate AT1R signaling in other brain regions that participate in the regulation of sympathetic nerve activity, including the anteroventral third ventricle, paraventricular nucleus of the hypothalamus, and nucleus tractus solitarii^{6,9}. Nonetheless, elimination of PHM effects on BP and urinary norepinephrine excretion by hydrogel introduction in RVLM (Fig. 6b–d) supports the critical role for RVLM. Because hydrogel may exert yet unknown effects, experiments of hydrogel introduction may not entirely prove the contribution of interstitial fluid movement. For example, hydrogel introduction may alter the stiffness and elasticity of extracellular matrix, which are known to affect the neurological physiology, pathology, and development⁵⁵. Although further studies are required to address these issues, our findings suggest that mechanical factors can be a therapeutic target within the blood-brain barrier, the accessibility to which of antihypertensive drugs appears variable and controversial⁵⁶.

Unlike the case of PHM in rats, VOCR of humans generates vertical accelerations at various body parts in addition to the head. Therefore, we cannot preclude the possibility that the antihypertensive effect of VOCR is mediated by mechanical regulation of tissues and organs other than the brain. Furthermore, our clinical studies of protocol 1 and 2 are based on a small number of subjects (Supplementary Table 2) with a fixed condition (frequency of 2 Hz, peak acceleration of ~1.0 x g, 30 minutes/day, 3 days/week, 12–14 rides), and need further analysis with a much larger sample size and varying conditions to determine optimal VOCR. Still, application of vertical

mechanical impacts with moderate magnitudes is expected to be highly safe with minimal possibility of adverse effects, providing a novel therapeutic/preventative strategy for physical disorders including those that are resistant to conventional treatments such as drug administration. Notably, our approach utilizing mechanical interventions may bring considerable benefits to those who cannot receive benefits from exercise because of physical disabilities.

Data availability

All data are included in this article and its supplementary information files. Raw data are available from the corresponding author upon reasonable request.

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Author contributions

S.M. and N.S. conducted most of the experiments. Y.S. conceived the research, designed the study, and led the project with help from M.N. and M.S. T.K. provided technical advice for all the experiments involving measurement of cardiovascular parameters. S.M., N.S., T.K., M.S. and Y.S. wrote the manuscript. T.M. and A.T. contributed to the design and construction of the machine for PHM. D.Y. helped in vitro FSS experiments and carried out simulative calculation of in vivo FSS. T.S. and Y.Y. developed and provided the PEG hydrogel system. A.K. and H.H. prepared and provided AAV vectors. K.T., T.K., M.A., H.I., and T.O. contributed to the human studies. K.S., T.S., S.T. M.S., T.O., and M.N. provided technical, advisory and financial support.

Competing interests

The authors declare no competing interest. S.M., T.M., T.O., A.T., and Y.S. joined the application of a patent for the vertically oscillating chair, which has been awarded in Japan (JP6592834) and filed internationally (US16/616,935; EP18806753.2; CN201880033284.0; IN201927048891).

References

1. Lim, S.S., *et al.* A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* **380**, 2224-2260 (2012).
2. Hagberg, J.M., Park, J.J. & Brown, M.D. The role of exercise training in the treatment of hypertension: an update. *Sports Med* **30**, 193-206 (2000).
3. Pescatello, L.S., MacDonald, H.V., Lamberti, L. & Johnson, B.T. Exercise for hypertension: a prescription update integrating existing recommendations with emerging research. *Curr Hypertens Rep* **17**, 87 (2015).
4. Carretero, O.A. & Oparil, S. Essential hypertension. Part I: definition and etiology. *Circulation* **101**, 329-335 (2000).
5. Guyton, A.C. Abnormal renal function and autoregulation in essential hypertension. *Hypertension* **18**, III49-53 (1991).
6. Guyenet, P.G. The sympathetic control of blood pressure. *Nat Rev Neurosci* **7**, 335-346 (2006).
7. Grassi, G., Mark, A. & Esler, M. The sympathetic nervous system alterations in human hypertension. *Circ Res* **116**, 976-990 (2015).
8. Malpas, S.C. Sympathetic nervous system overactivity and its role in the development of cardiovascular disease. *Physiol Rev* **90**, 513-557 (2010).
9. Dampney, R.A. Functional organization of central pathways regulating the cardiovascular system. *Physiol Rev* **74**, 323-364 (1994).
10. Fyhrquist, F. & Saijonmaa, O. Renin-angiotensin system revisited. *J Intern Med* **264**, 224-236 (2008).
11. Forrester, S.J., *et al.* Angiotensin II signal transduction: an update on mechanisms of physiology and pathophysiology. *Physiol Rev* **98**, 1627-1738 (2018).
12. Ito, S., Komatsu, K., Tsukamoto, K., Kanmatsuse, K. & Sved, A.F. Ventrolateral medulla AT1 receptors support blood pressure in hypertensive rats. *Hypertension* **40**, 552-559 (2002).
13. Muratani, H., Ferrario, C.M. & Averill, D.B. Ventrolateral medulla in spontaneously hypertensive rats: role of angiotensin II. *Am J Physiol* **264**, R388-395 (1993).
14. Kishi, T., *et al.* Exercise training causes sympathoinhibition through antioxidant effect in the rostral ventrolateral medulla of hypertensive rats. *Clin Exp Hypertens* **34**, 278-283 (2012).
15. Nabika, T., Ohara, H., Kato, N. & Isomura, M. The stroke-prone spontaneously hypertensive rat: still a useful model for post-GWAS genetic studies? *Hypertens Res* **35**, 477-484 (2012).
16. Lu, X., *et al.* Effects of local mechanical stimulation on coronary artery endothelial function and angiotensin II type 1 receptor in pressure or flow-overload. *J Hypertens* **31**, 720-729 (2013).

17. Galie, P.A., Russell, M.W., Westfall, M.V. & Stegemann, J.P. Interstitial fluid flow and cyclic strain differentially regulate cardiac fibroblast activation via AT1R and TGF- β 1. *Exp Cell Res* **318**, 75-84 (2012).
18. Zou, Y., *et al.* Mechanical stress activates angiotensin II type 1 receptor without the involvement of angiotensin II. *Nat Cell Biol* **6**, 499-506 (2004).
19. Ramkhalawon, B., *et al.* Shear stress regulates angiotensin type 1 receptor expression in endothelial cells. *Circ Res* **105**, 869-875 (2009).
20. Li, E.C., Heran, B.S. & Wright, J.M. Angiotensin converting enzyme (ACE) inhibitors versus angiotensin receptor blockers for primary hypertension. *Cochrane Database Syst Rev*, CD009096 (2014).
21. Weinbaum, S., Cowin, S.C. & Zeng, Y. A model for the excitation of osteocytes by mechanical loading-induced bone fluid shear stresses. *J Biomech* **27**, 339-360 (1994).
22. Tatsumi, S., *et al.* Targeted ablation of osteocytes induces osteoporosis with defective mechanotransduction. *Cell Metab* **5**, 464-475 (2007).
23. Miyazaki, T., *et al.* Mechanical regulation of bone homeostasis through p130Cas-mediated alleviation of NF- κ B activity. *Sci Adv* **5**, eaau7802 (2019).
24. Ryu, Y., *et al.* Mechanical regulation underlies effects of exercise on serotonin-induced signaling in the prefrontal cortex neurons. *iScience* **23**, 100874 (2020).
25. Minami, N., *et al.* Effects of exercise and β -blocker on blood pressure and baroreflexes in spontaneously hypertensive rats. *Am J Hypertens* **16**, 966-972 (2003).
26. Kim, S.E., *et al.* Treadmill exercise prevents aging-induced failure of memory through an increase in neurogenesis and suppression of apoptosis in rat hippocampus. *Exp Gerontol* **45**, 357-365 (2010).
27. Bertagnolli, M., *et al.* Exercise training reduces sympathetic modulation on cardiovascular system and cardiac oxidative stress in spontaneously hypertensive rats. *Am J Hypertens* **21**, 1188-1193 (2008).
28. Chidsey, C.A., Braunwald, E. & Morrow, A.G. Catecholamine excretion and cardiac stores of norepinephrine in congestive heart failure. *Am J Med* **39**, 442-451 (1965).
29. Mullen, R.J., Buck, C.R. & Smith, A.M. NeuN, a neuronal specific nuclear protein in vertebrates. *Development* **116**, 201-211 (1992).
30. Freeman, M.R. Specification and morphogenesis of astrocytes. *Science* **330**, 774-778 (2010).
31. Tamura, K., *et al.* The physiology and pathophysiology of a novel angiotensin receptor-binding protein ATRAP/Agtrap. *Curr Pharm Des* **19**, 3043-3048 (2013).
32. Huda, F., *et al.* Distinct transduction profiles in the CNS via three injection routes of AAV9 and the application to generation of a neurodegenerative mouse model. *Mol Ther Methods Clin Dev* **1**, 14032 (2014).

33. Tang, X., *et al.* "Self-cleaving" 2A peptide from porcine teschovirus-1 mediates cleavage of dual fluorescent proteins in transgenic *Eimeria tenella*. *Vet Res* **47**, 68 (2016).
34. Tworkoski, E., Glucksberg, M.R. & Johnson, M. The effect of the rate of hydrostatic pressure depressurization on cells in culture. *PLoS One* **13**, e0189890 (2018).
35. Saitou, K., *et al.* Local cyclical compression modulates macrophage function in situ and alleviates immobilization-induced muscle atrophy. *Clin Sci (Lond)* **132**, 2147-2161 (2018).
36. Maneshi, M.M., *et al.* Mechanical stress activates NMDA receptors in the absence of agonists. *Sci Rep* **7**, 39610 (2017).
37. Gong, S., *et al.* A gene expression atlas of the central nervous system based on bacterial artificial chromosomes. *Nature* **425**, 917-925 (2003).
38. Yoshino, D., Sakamoto, N., Takahashi, K., Inoue, E. & Sato, M. Development of Novel Flow Chamber to Study Endothelial Cell Morphology: Effects of shear flow with uniform spatial gradient on distribution of focal adhesion. *J Biomech Sci Eng* **8**, 233-243 (2013).
39. Goshima, Y., Ohsako, S. & Yamauchi, T. Overexpression of Ca²⁺/calmodulin-dependent protein kinase II in Neuro2A and NG108-15 neuroblastoma cell lines promotes neurite outgrowth and growth cone motility. *J Neurosci* **13**, 559-567 (1993).
40. Yun, J., *et al.* Neuronal Per Arnt Sim (PAS) domain protein 4 (NPAS4) regulates neurite outgrowth and phosphorylation of synapsin I. *J Biol Chem* **288**, 2655-2664 (2013).
41. Fujiyabu, T., Toni, F., Li, X., Chung, U.I. & Sakai, T. Three cooperative diffusion coefficients describing dynamics of polymer gels. *Chem Commun (Camb)* **54**, 6784-6787 (2018).
42. Jennings, J.R., Muldoon, M.F. & Sved, A.F. Is the brain an early or late component of essential hypertension? *Am J Hypertens* (2020).
43. Joyner, M.J. & Green, D.J. Exercise protects the cardiovascular system: effects beyond traditional risk factors. *J Physiol* **587**, 5551-5558 (2009).
44. Jancovski, N., *et al.* Angiotensin type 1A receptor expression in C1 neurons of the rostral ventrolateral medulla contributes to the development of angiotensin-dependent hypertension. *Exp Physiol* **99**, 1597-1610 (2014).
45. Guo, F., *et al.* Astroglia are a possible cellular substrate of angiotensin(1-7) effects in the rostral ventrolateral medulla. *Cardiovasc Res* **87**, 578-584 (2010).
46. Liddel, S.A. & Barres, B.A. Reactive astrocytes: production, function, and therapeutic potential. *Immunity* **46**, 957-967 (2017).
47. MacLulich, A.M., *et al.* Higher systolic blood pressure is associated with increased water diffusivity in normal-appearing white matter. *Stroke* **40**, 3869-3871 (2009).
48. Bedussi, B., *et al.* Enhanced interstitial fluid drainage in the hippocampus of spontaneously hypertensive rats. *Sci Rep* **7**, 744 (2017).
49. Hahn, C. & Schwartz, M.A. Mechanotransduction in vascular physiology and atherogenesis. *Nat Rev Mol Cell Biol* **10**, 53-62 (2009).

50. Elton, T.S. & Martin, M.M. Angiotensin II type 1 receptor gene regulation: transcriptional and posttranscriptional mechanisms. *Hypertension* **49**, 953-961 (2007).
51. Galie, P.A., van Oosten, A., Chen, C.S. & Janmey, P.A. Application of multiple levels of fluid shear stress to endothelial cells plated on polyacrylamide gels. *Lab Chip* **15**, 1205-1212 (2015).
52. Kadohama, T., Nishimura, K., Hoshino, Y., Sasajima, T. & Sumpio, B.E. Effects of different types of fluid shear stress on endothelial cell proliferation and survival. *J Cell Physiol* **212**, 244-251 (2007).
53. Iskratsch, T., Wolfenson, H. & Sheetz, M.P. Appreciating force and shape-the rise of mechanotransduction in cell biology. *Nat Rev Mol Cell Biol* **15**, 825-833 (2014).
54. Hughes, J.W., Watkins, L., Blumenthal, J.A., Kuhn, C. & Sherwood, A. Depression and anxiety symptoms are related to increased 24-hour urinary norepinephrine excretion among healthy middle-aged women. *J Psychosom Res* **57**, 353-358 (2004).
55. Li, S.C., *et al.* Tissue elasticity bridges cancer stem cells to the tumor microenvironment through microRNAs: implications for a "watch-and-wait" approach to cancer. *Curr Stem Cell Res Ther* **12**, 455-470 (2017).
56. Kishi, T., Hirooka, Y. & Sunagawa, K. Sympathoinhibition caused by orally administered telmisartan through inhibition of the AT(1) receptor in the rostral ventrolateral medulla of hypertensive rats. *Hypertens Res* **35**, 940-946 (2012).
57. Huang, N. & Bonn, D. Viscosity of a dense suspension in Couette flow. *J Fluid Mech* **590**, 497-507 (2007).
58. Sugiyama, S., *et al.* Computational simulation of convection-enhanced drug delivery in the non-human primate brainstem: a simple model predicting the drug distribution. *Neurol Res* **35**, 773-781 (2013).
59. Yao, W., Shen, Z. & Ding, G. Simulation of interstitial fluid flow in ligaments: comparison among Stokes, Brinkman and Darcy models. *Int J Biol Sci* **9**, 1050-1056 (2013).
60. Tarbell, J.M. & Shi, Z.-D. Effect of the glycocalyx layer on transmission of interstitial flow shear stress to embedded cells. *Biomech Model Mechanobiol* **12**, 111-121 (2013).
61. Sakai, K., *et al.* Overexpression of eNOS in NTS causes hypotension and bradycardia in vivo. *Hypertension* **36**, 1023-1028 (2000).
62. Kishi, T., *et al.* Overexpression of eNOS in the RVLM causes hypotension and bradycardia via GABA release. *Hypertension* **38**, 896-901 (2001).
63. Paxinos, G. & Watson, C. *The rat brain in stereotaxic coordinates* (Academic Press, 1998).
64. Hirooka, Y., Polson, J.W. & Dampney, R.A. Pressor and sympathoexcitatory effects of nitric oxide in the rostral ventrolateral medulla. *J Hypertens* **14**, 1317-1324 (1996).
65. Shinohara, Y., *et al.* Effects of neutralizing antibody production on AAV-PHP.B-mediated transduction of the mouse central nervous system. *Mol Neurobiol* **56**, 4203-4214 (2019).

66. Shinohara, Y., Ohtani, T., Konno, A. & Hirai, H. Viral vector-based evaluation of regulatory regions in the neuron-specific enolase (NSE) promoter in mouse cerebellum in vivo. *Cerebellum* **16**, 913-922 (2017).
67. Konno, A., *et al.* Mutant ataxin-3 with an abnormally expanded polyglutamine chain disrupts dendritic development and metabotropic glutamate receptor signaling in mouse cerebellar Purkinje cells. *Cerebellum* **13**, 29-41 (2014).
68. Matsuzaki, Y., *et al.* Neurotropic Properties of AAV-PHP.B are shared among diverse inbred strains of mice. *Mol Ther* **27**, 700-704 (2019).
69. Sakitani, N., *et al.* Application of consistent massage-like perturbations on mouse calves and monitoring the resulting intramuscular pressure changes. *J Vis Exp* e59475 (2019).
70. Liu, C., De Luca, A., Rosso, A. & Talon, L. Darcy's law for yield stress fluids. *Phys Rev Lett* **122**, 245502 (2019).
71. Schildge, S., Bohrer, C., Beck, K. & Schachtrup, C. Isolation and culture of mouse cortical astrocytes. *J Vis Exp* e50079 (2013).
72. Umemura, S., *et al.* The Japanese Society of Hypertension guidelines for the management of hypertension (JSH 2019). *Hypertens Res* **42**, 1235-1481 (2019).

Figures and figure legends

Fig. 1

Fig. 1 | Application of cyclical mechanical impact to the head by passive motion lowers the BP

599 **in SHRSPs, and AT1R expression in their RVLM astrocytes. a,** Schematic representation of
 600 experimental protocol for analysis of the effects of PHM on BP in rats. **b,c,** Time courses (**b**) and
 601 values on Day 29 (**c**) of MAP of WKY rats and SHRSPs, subjected to either daily PHM or
 602 anesthesia only (**b**: $P = 0.0814$ for Day15, $P = 0.0052$ for Day 22, and $P = 0.0046$ for Day 29. **c**: P
 603 $= 0.9739$ for column 1 vs. 2 and $P = 0.0046$ for column 3 vs. 4. $n = 7$ rats for each group of WKY
 604 and $n = 8$ rats for each group of SHRSP). **d,** HR values on Day 29 ($P = 0.9650$ for column 1 vs. 2
 605 and $P = 0.2362$ for column 3 vs. 4. $n = 7$ rats for each group of WKY and $n = 8$ rats for each group
 606 of SHRSP). **e,** Relative heart weight (heart weight / whole body weight) measured on Day 30 [$P =$
 607 0.9866 for column 1 vs. 2 and $P = 0.0152$ for column 3 vs. 4. $n = 10$ rats for WKY, PHM (-); $n = 13$
 608 rats for WKY, PHM (+); $n = 10$ rats for SHRSP, PHM (-); $n = 14$ rats for SHRSP, PHM (+)]. **f,**
 609 Twenty-four-hour (Day 29 to Day 30) urinary norepinephrine excretion [$P = 0.9854$ for column 1
 610 vs. 2 and $P = 0.0085$ for column 3 vs. 4. $n = 8$ rats for each group of WKY; $n = 16$ rats for SHRSP,
 611 PHM (-); $n = 13$ rats for SHRSP, PHM (+)]. **g,h,** Micrographic images of anti-NeuN (blue), anti-
 612 GFAP (green) and anti-AT1R (red) immunostaining of the RVLM of WKY rats (**g**) and SHRSPs
 613 (**h**), either left sedentary (top) or subjected to PHM (bottom) under anesthesia (30 minutes/day, 28
 614 days). Higher magnification images (center and right) refer to the areas indicated by dotted
 615 rectangles in low magnification images (left). Arrows point to anti-AT1R immunosignals that
 616 overlap with anti-GFAP, but not anti-NeuN, immunosignals in merged images. Scale bars, 50 μm .

617 Images are representative of three rats. **i,j**, Quantification of AT1R-positive neurons (**i**) and
618 astrocytes (**j**) in the RVLM of WKY rats and SHRSPs, either left sedentary or subjected to PHM.
619 Fifty NeuN-positive (NeuN⁺) cells and one hundred GFAP-positive (GFAP⁺) cells were analyzed
620 for each rat (**i**: $P = 0.7803$. **j**: $P = 0.9455$ for column 1 vs. 2, $P = 0.0004$ for column 1 vs. 3, and $P =$
621 0.0002 for column 3 vs. 4. $n = 3$ rats for each group). Data are presented as mean $\pm$ s.e.m. $*P < 0.05$;
622 $**P < 0.01$; $***P < 0.001$; NS, not significant; one-way ANOVA with Tukey's post hoc multiple
623 comparisons test.

Extended Data Fig. 1

Extended Data Fig. 1 | Daily anesthesia alone does not affect BP, and treadmill running

decreases AT1R expression in the RVLM astrocytes in SHRSPs. a, Time courses of MAP of

SHRSPs either routinely kept in cages (no anesthesia) or subjected to daily anesthesia on the platform of PHM machine without turning on its switch [PHM (-)] [$n = 6$ rats for no daily anesthesia and $n = 8$ rats for PHM (-)]. **b**, Micrographic images anti-NeuN (blue), anti-GFAP (green) and anti-AT1R (red) immunostaining of the RVLM of SHRSPs, either placed in the static treadmill machine or subjected to treadmill running at the velocity of 20 m/minute (30 minutes/day, 28 days). Arrows point to anti-AT1R immunosignals that overlap with anti-GFAP, but not anti-NeuN, immunosignals in merged images. Scale bar, 50 μ m. Images are representative of three or four rats. **c,d**, Quantification of AT1R-positive neurons (**c**) and astrocytes (**d**) in the RVLM of SHRSPs with or without 4-week treadmill running. Fifty NeuN⁺ cells and one hundred GFAP⁺ cells were analyzed for each rat [**c**: $P = 0.7056$. **d**: $P = 0.0048$. $n = 3$ rats for treadmill (-) and $n = 4$ rats for treadmill (+)]. Data are presented as mean $\pm$ s.e.m. $**P < 0.01$; NS, not significant, unpaired two-tailed Student's t -test.

Fig. 2

Fig. 2 | PHM alleviates the sensitivity of RVLM in SHRSPs to Ang II or valsartan. a,

Schematic representation of experimental protocol for analysis of the effects of PHM on the

sensitivity to Ang II or valsartan injected into unilateral RVLM. Ang II (100 pmol) was injected into

643 the unilateral RVLM of WKY rats and SHRSPs, either left sedentary (daily anesthesia) or subjected
644 to PHM (30 minutes/day, 28 days), with their BP monitored under urethane anesthesia. Injection of
645 valsartan (100 pmol) into the RVLM was conducted at least 2 hours after the injection of Ang II. **b**,
646 Representative trajectories of BP (top in each panel) and MAP (bottom in each panel). Arrows point
647 to the time of the initiation of RVLM injection of Ang II. **c**, Quantification of MAP change caused
648 by Ang II injection [$P = 0.9876$ for column 1 vs. 2 and $P = 0.0003$ for column 3 vs. 4. $n = 5$ rats for
649 each group of WKY; $n = 7$ rats for SHRSP, PHM (-); $n = 8$ rats for SHRSP, PHM (+)]. **d,e**, Effects
650 of RVLM injection of valsartan (100 pmol) examined as in **(b,c)** [**e**: $P = 0.9953$ for column 1 vs. 2
651 and $P = 0.0099$ for column 3 vs. 4. $n = 3$ rats for WKY, PHM (-); $n = 5$ rats for WKY, PHM (+); n
652 = 4 rats for each group of SHRSP]. Data are presented as mean $\pm$ s.e.m. $**P < 0.01$; $***P < 0.001$;
653 NS, not significant; one-way ANOVA with Tukey's post hoc multiple comparisons test.

Fig. 3

Fig. 3 | AAV-mediated expression of AGTRAP in RVLM astrocytes, but not neurons, lowers

656 **the BP in SHRSPs. a,** Schematic representation of experimental protocol for analysis of the effects
 657 of AAV-mediated transduction of RVLM astrocytes or neurons with AGTRAP gene. ITR; inverted
 658 terminal repeat. **b,c,** Astrocyte- (**b**) and neuron- (**c**) specific transgene expression by RVLM
 659 injection of AAV9 vectors. Micrographic images of GFP (green) and anti-GFAP (**b**) or anti-NeuN
 660 (**c**) immunostaining (blue) of RVLM of SHRSPs 15 days after the injection of AAV9 vectors
 661 indicated at the top of each panel. Scale bars, 50 μ m. Images are representative of three rats. **d–f,**
 662 Time courses (**d**) and values on Day 15 (**e**) of MAP (**d**: $P = 0.0229$ for Day 14. **e**: $P = 0.0229$ for
 663 column 1 vs. 2 and $P = 0.6864$ for column 3 vs. 4. $n = 6$ rats for GFAP-control; $n = 7$ rats for
 664 GFAP-AGTRAP; $n = 7$ rats for NSE-control; $n = 6$ rats for NSE-AGTRAP), and 24-hour urinary
 665 norepinephrine excretion (**f**) ($P = 0.0497$ for column 1 vs. 2 and $P = 0.7455$ for column 3 vs. 4. $n =$
 666 4 rats for each group) of SHRSPs subjected to RVLM injection of AAV9 vectors. Data are
 667 presented as mean $\pm$ s.e.m. $*P < 0.05$; NS, not significant, one-way ANOVA with Tukey's post hoc
 668 multiple comparisons test.

Extended Data Fig. 2

Extended Data Fig. 2 | AAV-mediated transduction of RVLM astrocytes or neurons in

SHRSPs. a, Schematic representation of injection of AAV9 vectors to RVLM. Micrographic image
 is representative of three rats analyzed in Fig. 3b (15 days after the RVLM injection of GFAP-
 AGTRAP vector). GFP-derived fluorescence indicates cells expressing the transgene. Scale bar, 1
 mm. **b,c,** Efficiency and specificity of astrocyte-specific expression of the transgene. **(b)**
 Micrographic images of GFP (green) and anti-NeuN immunostaining (blue) of RVLM of SHRSPs
 analyzed in Fig. 3b. Scale bar, 50 μ m. Images are representative of three rats. **(c)** Quantification of
 the efficiency and specificity of transgene expression. The relative populations (%) of GFP- GFAP-
 double positive (GFP⁺ GFAP⁺) or GFP- NeuN-double positive (GFP⁺ NeuN⁺) cells were calculated
 by referring their numbers to those of GFP⁺, GFAP⁺ or NeuN⁺ cells ($P < 0.0001$ for column 1 vs. 2
 and $P < 0.0001$ for column 3 vs. 4. $n = 3$ rats for each group). **d,e,** Efficiency and specificity of
 neuron-specific expression of the transgene. **(d)** Micrographic images of GFP (green) and anti-
 GFAP immunostaining (blue) of RVLM of SHRSPs analyzed in Fig. 3c. Scale bar, 50 μ m. Images
 are representative of three rats. **(e)** The efficiency and specificity quantified as in **(c)** ($P < 0.0001$ for
 column 1 vs. 2 and $P < 0.0001$ for column 3 vs. 4.). **f,** BP in SHRSPs injected with control vectors.
 BP was measured and MAP was quantified as in Fig. 1b ($P = 0.9803$ for Week 12, $P = 0.5914$ for
 Week 13, and $P = 0.4693$ for Week 14. $n = 6$ rats for no AAV injection; $n = 6$ rats for GFAP-control;
 $n = 7$ rats for NSE-control). The data for blue line are identical with those demonstrated with blue
 line in Extended Data Fig. 1a. Data are presented as mean $\pm$ s.e.m. *** $P < 0.001$; NS, not

689 significant; unpaired two-tailed Student's *t*-test **(c,e)** or one-way ANOVA with Tukey's post hoc
690 multiple comparisons test **(f)**.

Fig. 4

Fig. 4 | PHM generates pressure waves of low amplitude, but facilitates interstitial fluid

693 **movement (flow) in rat RVLM. a**, Schematic representation of pressure measurement in rat
 694 RVLM. **b**, Representative pressure waves recorded in rat RVLM during sedentary condition and
 695 PHM. Arrows point to the time of transition from inhalation to exhalation detected by simultaneous
 696 respiration monitoring. Scale bar, 1 s. Images are representative of three independent experiments
 697 with similar results. **c**, Respiration-unsynchronized pressure changes. Respiration-synchronized
 698 pressure waves indicated by dotted rectangles in **(b)** are presented with high magnification. Right-
 699 angled scale bar, 1 s / 1 mm Hg. Note that 2-Hz pressure waves indicated by a two-headed dotted
 700 line arrow were specifically generated during PHM. **d**, Magnitude of PHM-specific and -unspecific
 701 pressure changes unsynchronized with respiration. Peak to peak magnitudes indicated by two-
 702 headed arrows in **(c)** were quantified ($P = 0.0089$. $n = 4$ rats for each group, 10 segments analyzed
 703 for each rat). **e**, Schematic representation of experimental protocol for μ CT analysis of Isovist
 704 injected in rat RVLM. **f**, Definition of x -(left-right), y -(rostral-caudal), and z -(dorsal-ventral) axes
 705 used in this study. **g**, Representative Isovist spreading presented on X-ray images. Isovist clusters
 706 are indicated by green. Images are representative of three rats. A dotted line arrow indicates the
 707 main direction of spreading in this sample. **h**, Quantification of Isovist spreading along each axis
 708 (left chart: $P = 0.6666$. middle chart: $P = 0.0218$. right chart: $P = 0.0244$. $n = 3$ rats for each group).
 709 Data are presented as mean $\pm$ s.e.m. * $P < 0.05$; ** $P < 0.01$; NS, not significant, unpaired two-tailed
 710 Student's t -test.

Supplementary Table 1

a

Property	Value
Pressure changes (ΔP ; mmHg)	1.19
Viscosity (μ ; mPa•s)	1–20 [#]
Spread distance along x-axis per each PHM cycle (Δx ; μm)	–0.002
Spread distance along y-axis per each PHM cycle (Δy ; μm)	0.039
Spread distance along z-axis per each PHM cycle (Δz ; μm)	0.055
Velocity of interstitial fluid flow along x-axis ($u_{\infty,x}$; $\mu\text{m/s}$)	–0.004
Velocity of interstitial fluid flow along y-axis ($u_{\infty,y}$; $\mu\text{m/s}$)	0.079
Velocity of interstitial fluid flow along z-axis ($u_{\infty,z}$; $\mu\text{m/s}$)	0.111

b

FSS (τ_x) along x-axis at the cell surface:

$$\tau_x = \frac{\mu u_{\infty,x}}{\sqrt{K_{p,x}}}$$

$$K_{p,x} = \frac{\mu u_{\infty,x} \Delta x}{\Delta P}$$

, where $K_{p,x}$ is the Darcy permeability of brain tissue along x-axis.

The shear stresses along y- and z-axes can be calculated in a similar manner.

When the values listed in **a** are introduced in these equations, the magnitude of FSS is estimated as 0.59–2.64 Pa.

Supplementary Table 1 | Simulative calculation of the magnitude of PHM-generated FSS on

rat RVLM cells. a, Values referenced for simulative calculation of the magnitude of FSS that PHM

generated in rat RVLM. All referenced values except viscosity (marked with #) were drawn from

715 analyses with IBP measurement and Isovist-enhanced μ CT scanning (Fig. 4d,h). The property of
716 interstitial fluid viscosity was referenced from previous studies⁵⁷⁻⁵⁹. **b**, Calculation of the magnitude
717 of FSS generated by PHM. FSS (τ) at the cell surface can be calculated as reported previously⁶⁰.

Fig. 5

Fig. 5 | FSS on cultured astrocytes decreases their AT1R expression and Ang II-binding

potential in vitro. a–c, AT1R expression in cultured astrocytes with or without exposure to FSS.

Astrocytes prepared from astrocyte-GFP mice, either left unexposed or exposed to pulsatile FSS

(average 0.7 Pa, 0.5 Hz, 30 minutes) were analyzed 6 and 24 hours after the cessation of 30-minute

FSS application. **(a)** AT1R mRNA expression levels normalized against GAPDH expression and

scaled as the mean value from control samples (cells left unexposed to FSS) set as 1 ($P = 0.0050$ for

column 1 vs. 2 and $P = 0.0011$ for column 1 vs. 3. $n = 6$). **(b)** Microscopic images of anti-AT1R (red) and anti-GFP (green) immunostaining. Images are representative of three or four independent experiments with similar results. Scale bar, 50 μm . **(c)** Relative population of AT1R- GFP-double positive (AT1R⁺ GFP⁺) cells were quantified as a ratio to total GFP-positive (GFP⁺) cells in each sample [$P = 0.0021$ for column 1 vs. 2 and $P = 0.0002$ for column 1 vs. 3. >100 GFP-positive cells were analyzed in each sample; $n = 4$ for FSS (-), $n = 3$ for 6 and 24 hours after FSS]. **d,e**, Effect of FSS on astrocytes' Ang II-binding potential. Cultured astrocytes were either left unexposed or exposed to FSS as in **(a-c)**. Six and twenty-four hours after the cessation of 30-minute FSS application, cells were subjected to fluorescent Ang II binding assay. **(d)** Microscopic images representative of three independent experiments with similar results. Scale bar, 50 μm . **(e)** GFP-positive cells with punctate red fluorescence (TAMRA-Ang II-bound astrocytes) were quantified as a ratio (%) to total GFP-positive cells in each sample ($P < 0.0001$ for column 1 vs. 2 and $P = 0.0001$ for column 1 vs. 3. 100 GFP-positive cells were analyzed in each sample; $n = 3$ for each group). Data are presented as mean $\pm$ s.e.m. $**P < 0.01$, $***P < 0.001$, one-way ANOVA with Tukey's post hoc multiple comparisons test.

Extended Data Fig. 3

Extended Data Fig. 3 | Preparation of astrocyte-enriched primary culture, and lack of decreasing effect of FSS on AT1R expression in cultured neuronal cells. **a**, Representative micrographic image of anti-GFP immunostaining (green) and DAPI staining (blue) of astrocyte-enriched culture prepared from astrocyte-GFP mice. Scale bar, 50 μ m. Note that most of the cells are GFP-positive. **b–d**, Effects of FSS on AT1R expression in Neuro2A cells analyzed as in Fig. 5a–c. **(b)** AT1R mRNA expression levels ($P = 0.2834$, $n = 4$). **(c)** Micrographic images representative of three independent experiments with similar results. Scale bar, 50 μ m. **(d)** Relative population of AT1R- TUJ-1-double positive (AT1R⁺ TUJ-1⁺) cells quantified as a ratio to total TUJ-

749 1-positive (TUG-1⁺) cells in each sample ($P = 0.2308$. >100 TUG-1-positive cells were analyzed in
750 each sample; $n = 3$). Data are presented as mean $\pm$ s.e.m. NS, not significant, one-way ANOVA with
751 Tukey's post hoc multiple comparisons test **(b)** or unpaired two-tailed Student's t -test **(d)**.

Fig. 6

Fig. 6 | Hydrogel introduction eliminates decreasing effects of PHM on BP and AT1R

expression in the RVLM astrocytes in SHRSPs. a, Schematic representation of experimental

755 protocol for analysis of the effects of PHM with and without PEG hydrogel introduction in bilateral
 756 RVLMs of SHRSPs. PHM was applied daily for consecutive 28 days. **b–d**, Time courses (**b**) and
 757 values on Day 29 (**c**) of MAP, and 24-hour urinary norepinephrine excretion (**d**) of SHRSPs,
 758 subjected to various combinations of daily PHM application and hydrogel introduction in bilateral
 759 RVLMs. Note the absence of significant difference in BP (**b,c**) and urinary norepinephrine excretion
 760 (**d**) of SHRSPs with hydrogel introduced RVLMs [Gel (+)] between with and without PHM [**b**: $P =$
 761 0.0372 for Day 29. **c**: $P = 0.0387$ for column 1 vs. 2, $P = 0.0372$ for column 1 vs. 3, and $P = 0.9959$
 762 for column 2 vs. 3. $n = 7$ rats for Gel (–), PHM (+) and for Gel (+), PHM (+); $n = 6$ rats for Gel (+),
 763 PHM (–); **d**: $P = 0.0247$ for column 1 vs. 2, $P = 0.0307$ for column 1 vs. 3, and $P = 0.9920$ for
 764 column 2 vs. 3. $n = 5$ rats for each group]. **e**, Micrographic images of anti-GFAP (green), anti-AT1R
 765 (red), and anti-NeuN (blue) immunostaining of the RVLM in SHRSPs subjected to various
 766 combinations of hydrogel introduction in bilateral RVLMs and 4-week PHM application. Arrows
 767 point to anti-AT1R immunosignals that overlap with anti-GFAP, but not anti-NeuN, immunosignals
 768 in merged images. Scale bar, 50 μm . Images are representative of four rats. **f,g**, Quantification of
 769 AT1R-positive astrocytes (**f**) and neurons (**g**) in the RVLM. Note the absence of significant
 770 difference in the ratio of AT1R-positive astrocytes of SHRSPs with hydrogel introduced RVLMs
 771 [Gel (+)] between with and without PHM (**f**, columns 2 and 3). Fifty NeuN⁺ cells and one hundred
 772 GFAP⁺ cells were analyzed for each rat (**f**: $P < 0.0001$ for column 1 vs. 2, $P < 0.0001$ for column 1

773 vs. 3, and $P = 0.1597$ for column 2 vs. 3. **g**: $P = 0.5182$. $n = 4$ rats for each group). Data are
774 presented as mean $\pm$ s.e.m. $*P < 0.05$; $***P < 0.001$; NS, not significant, one-way ANOVA with
775 Tukey's post hoc multiple comparisons test.

Extended Data Fig. 4

Extended Data Fig. 4 | Hydrogel introduction in the RVLM does not affect the depressor

response to valsartan or survival/apoptosis of RVLM astrocytes and neurons in SHRSPs. a,

Introduction of PEG hydrogel in rat RVLM. Twenty-four hours after the injection of control

ungelatable fluorescent PEG solution (left) or 1 week after the injection of pre-gel fluorescent PEG

781 solution (right), brainstem samples were prepared. Coronal-section images representative of three
 782 rats with similar results are shown. Scale bar, 1 mm. **b**, Schematic representation of experimental
 783 protocol. One week after the injection of pre-gel PEG solution or its ungelatable control, depressor
 784 response was analyzed as in Fig. 2. **c,d**, Representative trajectories (**c**) and quantification (**d**) of BP
 785 descent upon valsartan injection to the RVLM of SHRSPs with or without hydrogel introduction (**d**:
 786 $P = 0.2342$. $n = 3$ rats for each group). **e–j**, Effects of hydrogel introduction on survival/apoptosis
 787 of RVLM cells in SHRSPs. Rat RVLM sections prepared in the experiments for Fig. 6e–g [PHM
 788 (+), Gel (–) and PHM (+), Gel (–)] were subjected to TUNEL assay (**e,f**), or combinations of anti-
 789 GFAP, anti-NeuN and anti-cleaved caspase-3 immunostaining (**g–j**). DAPI-positive nuclei (**e**),
 790 GFAP- (**g**) or NeuN- (**i**) positive cells were counted, and the relative populations of cells doubly
 791 positive for indicated combinations of TUNEL (**f**) and cleaved caspase-3 (**h,j**) were quantified. Each
 792 value represents an average from five images of 1 x 1-mm area analyzed for each rat (**e**: $P = 0.6518$.
 793 **f**: $P = 0.6943$. **g**: $P = 0.7938$. **h**: $P = 0.8722$. **i**: $P = 0.1679$. **j**: $P = 0.7247$. $n = 3$ rats for each group).
 794 Data are presented as mean $\pm$ s.e.m. NS, not significant; unpaired two-tailed Student's *t*-test.

Fig. 7

795

796 Fig. 7 | VOCR has an antihypertensive effect on hypertensive adult humans. a, BP and HR

797 “value of the day”s immediately before and after 4-week VOCR in the study of protocol 1 (SBP: P
 798 = 0.0018. DBP: P = 0.1509. MAP: P = 0.0459. HR: P = 0.3900. n = 5). **b**, BP and HR “value of the
 799 week”s immediately before and after 4.5-week VOCR in the study of protocol 2 (SBP: P < 0.0001.
 800 DBP: P = 0.0031. MAP: P = 0.0009. HR: P = 0.9817. n = 15). **c**, Subject number-corresponding
 801 trajectories and statistical analysis of BP and HR in the study of protocol 2. Gray-shaded rectangles
 802 indicate the VOCR intervention periods (4.5 weeks). The colors and symbols of individual lines
 803 correspond to those of individual subject numbers listed on the right, excluding subject 9 (see
 804 Supplementary Table 2). Each “value of the week” was statistically compared with that of the week
 805 immediately before the initiation of VOCR intervention (SBP: P = 0.0293 for Pre vs. Week 1, P =
 806 0.00228 for Pre vs. Week 2, P = 0.0013 for Pre vs. Week 3, P = 0.0035 for Pre vs. Week 4, P =
 807 0.0002 for Pre vs. Week 5, P = 0.0078 for Pre vs. Week 6, P = 0.0035 for Pre vs. Week 7, P =
 808 0.0075 for Pre vs. Week 8, P = 0.2132 for Pre vs. Week 9, P = 0.1314 for Pre vs. Week 10, P =
 809 0.0973 for Pre vs. Week 11, and P = 0.3993 for Pre vs. Week 12. DBP: P = 0.3022 for Pre vs. Week
 810 1, P = 0.0436 for Pre vs. Week 2, P = 0.0010 for Pre vs. Week 3, P = 0.0100 for Pre vs. Week 4, P =
 811 0.0006 for Pre vs. Week 5, P = 0.0599 for Pre vs. Week 6, P = 0.0488 for Pre vs. Week 7, P =
 812 0.0096 for Pre vs. Week 8, P = 0.9346 for Pre vs. Week 9, P = 0.7850 for Pre vs. Week 10, P =
 813 0.0769 for Pre vs. Week 11, and P = 0.3137 for Pre vs. Week 12. MAP: P = 0.1075 for Pre vs. Week
 814 1, P = 0.0132 for Pre vs. Week 2, P = 0.0008 for Pre vs. Week 3, P = 0.0063 for Pre vs. Week 4, P =

815 0.0003 for Pre vs. Week 5, $P = 0.0251$ for Pre vs. Week 6, $P = 0.0136$ for Pre vs. Week 7, $P =$
 816 0.0071 for Pre vs. Week 8, $P = 0.6795$ for Pre vs. Week 9, $P = 0.4295$ for Pre vs. Week 10, $P =$
 817 0.0704 for Pre vs. Week 11, and $P = 0.3662$ for Pre vs. Week 12. HR: $P = 0.6287$ for Pre vs. Week
 818 1, $P = 0.7840$ for Pre vs. Week 2, $P = 0.1573$ for Pre vs. Week 3, $P = 0.5380$ for Pre vs. Week 4, $P =$
 819 0.7331 for Pre vs. Week 5, $P = 0.6995$ for Pre vs. Week 6, $P = 0.9110$ for Pre vs. Week 7, $P =$
 820 0.9875 for Pre vs. Week 8, $P = 0.5866$ for Pre vs. Week 9, $P = 0.9566$ for Pre vs. Week 10, $P =$
 821 0.6487 for Pre vs. Week 11, and $P = 0.9905$ for Pre vs. Week 12. $n = 15$ for Pre and Weeks 1 to 5, n
 822 $= 13$ for Week 6, $n = 12$ for Week 7, $n = 11$ for Weeks 8 and 9, $n = 9$ for Week 10, $n = 7$ for Week
 823 11, $n = 3$ for Week 12). $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; NS, not significant, paired two-tailed
 824 Student's t -test.

Extended Data Fig. 5

825

826 **Extended Data Fig. 5 | Vertically oscillating chair that reproduces mechanical impacts on the**

827 **head during light jogging, and protocols of clinical study to examine antihypertensive effect of**
828 **VOCR on hypertensive adult humans. a,** Vertical accelerations generated at adult human head
829 during light jogging on a treadmill machine (velocity: 7 km/hour) and VOCR (frequency: 2 Hz).
830 The VOCR system was adjusted to produce $\sim 1.0 \times g$ vertical acceleration peaks. Right-angled scale
831 bar, $0.5 \times g / 0.5 \text{ s}$. Images are representative of three independent experiments with similar results.
832 **b,** Photograph of the chair. **c,d,** Protocol 1 (**c**) and 2 (**d**).

Extended Data Fig. 6

833

834 **Extended Data Fig. 6 | Trajectories of BP and HR, and blood test values before and after the**

835 **intervention period in the study of protocol 2. a, Trajectories of “value of the week”s of BP and**

836 **HR. Gray-shaded rectangles indicate the VOICR intervention periods. The colors and symbols of**

837 **individual lines correspond to those of individual subjects (Fig. 7c). The black line with inverted**

838 **triangles indicates subject 9. b, Blood test values before and after the intervention period.**

839 Significant change was not observed in any of tested parameters. NS, not significant; paired two-
840 tailed Student's *t*-test. (Aldosterone: $P = 0.6265$. Renin activity: $P = 0.3794$. Epinephrine: $P =$
841 0.5103 . Norepinephrine: $P = 0.2653$. Dopamine: $P > 0.9999$. CRP: $P = 0.4412$.). A participant
842 (subject 18) showed a large increase in plasma renin activity after VOCR. We advised him to
843 consult his primary care physician, who ruled out the disqualifying conditions for this study (e.g.,
844 severe renal disease; see Methods) based on comprehensive evaluation. Therefore, we did not
845 exclude subject 18 from our statistical analysis of BP and HR.

Supplementary Table 2

Subject #	Sex	Period since diagnosis or self-recognition as hypertension	Smoking	Current medication (dose per day)	Habitual exercise just before (left) and after (right) (times per week)	SBP/DBP (mm Hg) and HR (bpm)	
	Age (years)					VOCR period (month of first - last bout of VOCR)	
	Body weight (kg)	Health problems and diseases other than hypertension	Alcohol (if yes, how often)				
	Height (cm)						
	BMI						
1	Male	13 years	No	Azilsartan • Amlodin (20 mg • 5 mg) Febuxostat (10 mg) Bisoprolol fumarate (5 mg)	Walking 90 minutes (3 or 4 times)	148/85	139/78
	60					62	66
	73						
	165.2	Hyperuricemia	Almost every day			February - March	
2	Female	14 years	No	None	Sit-ups 2 x 30 times (every day)	135/90	127/81
	53					70	69
	50						
	159	None	Almost every day			March	
3	Male	19 years	No	None	Judo (once)	135/86	129/91
	37					68	54
	113						
	166	None	Occasionally			March	
4	Female	6 years	No	None	None	141/87	132/80
	60					79	80
	73						
	156	None	Occasionally			April - May	
5	Female	1 year	No	Azilsartan (20 mg) Amlodin (2.5 mg)	None	144/84	140/80
	52					74	69
	50						
	148	None	Occasionally			April - May	
6	Female	10 years	No	None	None	128/70	109/70
	57					77	76
	68						
	164	None	Occasionally			November - December	
7	Male	Uncertain (<1 year)	No	Metformin hydrochloride (1000 mg) Rosuvastatin calcium (5 mg) Sitagliptin phosphate hydrate (50 mg)	Walking 120 minutes (once)	146/100	128/86
	61					92	84
	67	Diabetes mellitus	No				
	168	Hyperlipidemia				November - December	
8	Male	3 months	Yes	Montelukast sodium (10 mg) Ebastine (10 mg)	Walking 60 minutes (once) Stretching 30 minutes (once)	135/95	118/78
	46					61	59
	72						
	178	Allergic rhinitis	Occasionally			November - December	
9	Male	7 months	No	None	Karate (2 or 3 times)	156/89	145/94
	55					74	67
	87						
	173	Diabetes mellitus	No			November - December	
10	Male	6 months	No	None	Walking 40 minutes (4 times)	140/92	121/79
	70					81	89
	62						
	166	None	Occasionally			November - December	
11	Female	Uncertain (>1year)	No	Tamoxifen citrate (20 mg)	None	155/92	136/81
	60					74	67
	45						
	157	Breast cancer (post-surgery)	Almost every day			November - December	
12	Male	Uncertain (<1 year)	No	None	None	133/86	129/88
	68					70	70
	72						
	171	None	No			January - February	
13	Female	7 years	No	Telmisartan • Amlodipine (40 mg • 5 mg)	None	137/95	133/93
	56					65	71
	58						
	160	None	No			January - February	
14	Male	Uncertain (<1 year)	No	Sitagliptin phosphate hydrate (50 mg) Acetazolamide (250 mg)	None	140/90	124/77
	49					95	83
	69						
	168	Diabetes mellitus	No			January - February	
	24.5						

846

847

848

Subject #	Sex	Period since diagnosis or self-recognition as hypertension	Smoking	Current medication (dose per day)	Habitual exercise (times per week)	SBP/DBP (mm Hg) and HR (bpm) just before (left) and after (right) VOCR period (month of first - last bout of VOCR)			
	Age (years)								
	Body weight (kg)		Health problems and diseases other than hypertension			Alcohol (if yes, how often)			
	Height (cm)								
	BMI								
15	Male	Uncertain (<1 year)	No	None	None	140/87	124/75		
	43					76	62		
	69					None	Occasionally	August - September	
	165								
	25.3								
16	Female	15 years	No	None	Walking 60 minutes (6 times)	157/101	140/92		
	48					72	96		
	45.3					None	Occasionally	October - November	
	157.2								
	18.3								
17	Male	8 years	Yes	None	None	140/93	134/89		
	65					59	56		
	60.1					None	Almost every day	October	
	167.1								
	21.5								
18	Male	8 years	No	Azilsartan (20 mg) Lansoprazole (15 mg) Atorvastatin calcium hydrate (10 mg) Benidipine hydrochloride (4 mg)	None	172/123	115/78		
	55					69	88		
	93.4					Reflex esophagitis Hyperlipidemia	Almost every day	November - December	
	168.1								
	33.1								
19	Male	2 years	No	None	Walking 60 minutes (3 times)	130/83	127/89		
	41					64	56		
	92.3					None	Almost every day	January - February	
	183.2								
	27.5								
20	Female	1 year	No	Amlodipine besilate (5 mg) Atorvastatin calcium hydrate (10 mg) Limaprost alfadex (15 µg) Loxoprofen sodium hydrate (120 mg) Rebamipide (200 mg)	Swimming 40 minutes (5 times)	136/87	118/75		
	65					70	67		
	48.7					Hyperlipidemia Lumbar spinal canal stenosis (post-surgery)	Almost every day	January - February	
	158.6								
	19.4								
21	Male	2 years	No	None	None	163/109	126/86		
	32					71	80		
	65.1					None	Almost every day	January - February	
	168.9								
	22.8								

Supplementary Table 2 | Information of subjects who participated in the human studies.

Subjects 1–5 were participants in the study of protocol 1, and subjects 6–21 participated in the study of protocol 2. Subject 9 was excluded from our statistical analysis because of high serum CRP value before VOCR (2.85 mg/dL), which made it difficult to rule out acute infection, a possible disqualifier, at the time of initiation of VOCR, albeit the lack of specific complaint or local symptom related to acute physical problem(s). His serum CRP after the VOCR period was within the normal range (0.12 mg/dL).

Extended Data Fig. 7

857

858 **Extended Data Fig. 7 | Schematic representation of the antihypertensive effects observed in**

859 **PHM of hypertensive rats and VOCR of hypertensive humans.** The results from our animal
860 experiments indicate that cyclical mechanical impact on the head generates interstitial fluid
861 movement in the RVLM, leading to FSS-induced decrease in AT1R expression in astrocytes in situ,
862 and thereby ameliorates hypertension. Our studies also show that the VOCR of hypertensive adult
863 humans, which produces vertical accelerations at their heads, lowers their BP.

864 **Methods**

865 **Animal experiments and human studies.** Animals were housed under a 12/12 hour light-dark
866 cycle with controlled temperature (23–25°C), and treated with humane care under approval from
867 the Animal Care and Use Committee of National Rehabilitation Center for Persons with Disabilities
868 (approval number: 30-07). Male SHRSP/Izm and WKY/Izm rats were provided from the Disease
869 Model Cooperative Association (Kyoto, Japan) and astrocyte-GFP mice (Aldh1L1-GFP mice)³⁷
870 were obtained from GENSAT (New York, NY), acclimated to the laboratory environments for at
871 least 1 week, randomly divided into experimental groups, and used for experiments.

872 All participants in our human studies provided written informed consent. The studies were
873 approved by the Ethics Committees of the Iwai Medical Foundation and the National Rehabilitation
874 Center for Persons with Disabilities (approval number: 30-01).

875

876 **Chemicals and antibodies.** All the chemicals were purchased from Sigma-Aldrich unless noted
877 otherwise. Primary antibodies and their dilution rates used in this study are as follows: mouse
878 monoclonal anti-GFAP (MAB360; Millipore, Billerica, MA) at 1:1,000; rabbit polyclonal anti-
879 GFAP (Z0334; Dako, Glostrup, Denmark) at 1:1,000; chicken polyclonal anti-GFAP (ab4674;
880 Abcam, Cambridge, UK) at 1:2,000; rabbit polyclonal anti-cleaved caspase-3 (9661; Cell Signaling
881 Technology, Danvers, MA) at 1:1,000; mouse monoclonal anti-NeuN (MAB377; Millipore) at

882 1:200; rabbit polyclonal anti-NeuN (ABN78; Millipore) at 1:1,000, rabbit polyclonal anti-AT1R
883 (HPA003596; Sigma-Aldrich, St. Louis, MO) at 1:200; rabbit polyclonal anti-AGTRAP
884 (HPA044120; Sigma-Aldrich) at 1:1000; rabbit polyclonal anti-GFP (598; MBL, Nagoya, Japan) at
885 1:2,000; chicken polyclonal anti-GFP (ab13970; Abcam) at 1:2,000; mouse monoclonal anti-TUJ-1
886 (ab78078; Abcam) at 1:1000. Secondary antibodies conjugated with Alexa Fluor 350, 488, 568,
887 633, and 647 (Thermo Fisher Scientific, Waltham, MA) were used at the dilution rate of 1:400. Cell
888 nuclei were stained with DAPI (D9542; Sigma-Aldrich).

889

890 **PHM application to rats.** Rats were subjected to PHM in a prone position using a platform that we
891 developed to move their heads up and down²⁴ (schematically represented in Fig. 1a). During PHM,
892 animals were kept anesthetized with 1.5% isoflurane except for the μ CT study, in which we used
893 intraperitoneal injection of 2 mg/kg of midazolam (Sandoz, Basel, Switzerland), 2.5 mg/kg of
894 butorphanol (Meiji Seika, Tokyo, Japan) and 0.15 mg/kg of medetomidine (Kyoritsu Seiyaku,
895 Tokyo, Japan) for anesthesia. Body temperature of tested animals was maintained using a light
896 heater. The PHM system was set up to reproduce the head motion (5 mm, 2 Hz) of treadmill
897 running (20 m/minute) which made 1.0 x g vertical acceleration peaks at the head of rats
898 examined²⁴. The control rats in PHM experiments were anesthetized likewise, and placed in a prone
899 position with their heads on the platform that was left static.

900

901 **Treadmill running of rats.** Rats were subjected to compulsive running using a belt drive treadmill
902 equipped with an electrical shock system (MK-680S; Muromachi, Tokyo, Japan). We habituated the
903 rats to the treadmill system by placing them in the machine several times without turning on the
904 treadmill belt during the acclimation period. The electrical stimulation was turned on only once or
905 twice during the first 5 minutes of the 30-minute treadmill running on the first day of the 4-week
906 treadmill running period. Thereafter, we did not need to turn on the electrical shock system to have
907 the animals keep running, perhaps because the velocities we employed (20 m/minute) were
908 moderate. The control rats in treadmill running experiments were placed on the belt daily for 30
909 minutes without turning on the treadmill.

910

911 **Measurement of BP and HR of rats by radio-telemetry.** Rats were implanted with a telemetry
912 pressure probe (Millar, Houston, TX) in the abdominal aorta at 9–10 weeks of age, following the
913 surgical procedure described previously⁶¹. Rats were allowed to recover for at least 14 days before
914 the initiation of experimental interventions or analyses. During the periods of experimentation that
915 involved repeated BP and HR measurements over multiple weeks, BP and HR were monitored and
916 recorded for continuous 30 minutes every 7 days between 10:00 AM and 12:00 PM (noon) by a
917 multichannel amplifier and signal converter (LabChart 8; ADInstruments, Dunedin, New Zealand).

918 Mean arterial pressure (MAP) was calculated with a standard formula as follows: $MAP = \text{diastolic BP (DBP)} + 1/3 [\text{systolic BP (SBP)} - \text{DBP}]$.

920

921 **Measurement of urinary norepinephrine excretion of rats.** Urine excreted during the indicated
922 24-hour period was collected by means of a metabolic cage (KN-646; Natsume Seisakusho, Tokyo,
923 Japan), and stored at -80°C until assayed. Excretion of urinary norepinephrine was calculated by
924 multiplying its concentration measured using an enzyme-linked immunosorbent assay (ELISA) kit
925 (KA1891; Abnova, Taipei, Taiwan) with the urine volume.

926

927 **Microinjection into rat RVLM.** Rats were anesthetized with intraperitoneal injection of
928 midazolam, butorphanol, and medetomidine except for Ang II or valsartan injection studies, in
929 which we used 1.2–1.4 g/kg of urethane (Sigma-Aldrich), and subjected to microinjection as
930 described previously⁶². In brief, a 25s-G microsyringe (Hamilton, Bonaduz, Switzerland) was
931 stereotaxically positioned on anesthetized rats after exposure of dorsal surface of medulla. The
932 needle placement was defined according to an atlas of the rat with stereotaxic coordinates⁶³;
933 anteroposterior angle: 18° , 1.8 mm lateral to the calamus scriptorius, 3.5 mm ventral to the dorsal
934 surface of the medulla. The placement of the needle tip in RVLM was confirmed by ensuring the
935 pressure response to a test-dose injection of L-glutamate^{12,64} (100 nL of 1 mmol/L in PBS).

Microinjection of various compounds or mediums was made through a needle reinserted at the same coordinates with fixed infusion rates using a microsyringe pump instrument (KD scientific, Holliston, MA). Except for experiments to analyze pressor or depressor responses, we held the syringe for 5 minutes after the injection to avoid reflux, pulled out the needle carefully, and sutured the skin. The volumes and rates of microinjection were as follows; Ang II (Auspep, Tullamarine, Australia) and valsartan (Tocris Bioscience, Bristol, UK): 100 nL of 1 mmol/L in PBS at 0.1 μ L/minute, AAV solutions: 300 nL at 0.03 μ L/minute, PEG solutions: 1 μ L at 0.1 μ L/minute, Isovist: 1 μ L at 0.2 μ L/minute.

Analysis of pressor/depressor responses. Rats implanted with a telemetry pressure probe were anesthetized with urethane, and subjected to the analysis of pressor/depressor responses. Monitoring the BP, we injected Ang II or valsartan (100 pmol) stereotaxically into the unilateral RVLM following the microinjection procedures described above. The injection side (right or left) was chosen randomly. When both pressor and depressor responses were analyzed, at least 2 hours elapsed between the injections of Ang II and valsartan (Fig. 2a). The maximal MAP change elicited from the baseline was statistically analyzed¹⁴.

Production of AAV vectors. To obtain astrocyte- and neuron-specific transduction, we used AAV9

vectors that expressed a transgene under the control of mouse GFAP and rat NSE promoters, respectively. The astrocyte-specific GFAP promoter consists of 0.6-kb hybrid fragments containing ABC1D genomic regions upstream of the mouse *GFAP* gene⁶⁵. The neuron-specific NSE promoter is composed of the 1.2-kb genomic region upstream of the rat *NSE* gene⁶⁶. Full-length rat AGTRAP cDNA was synthesized (Eurofins Genomics, Tokyo, Japan) and inserted into plasmid pAAV-GFAP-GFP-P2A-Cre-woodchuck hepatitis virus post-transcriptional regulatory element (WPRE)-simian virus 40 polyadenylation signal (SV40pA) and pAAV-NSE-GFP-P2A-Cre-WPRE-SV40pA to generate pAAV-GFAP-GFP-P2A-AGTRAP-WPRE-SV40pA and pAAV-NSE-GFP-P2A-AGTRAP-WPRE-SV40pA. pAAV-GFAP-GFP-WPRE-SV40pA and pAAV-NSE-GFP-WPRE-SV40pA were used for experimental controls. Recombinant single-strand AAV2/9 vectors were produced by transfection of HEK293T cells (Thermo Fisher Scientific) with the respective pAAV expression plasmid, pAAV2/9 and a helper plasmid (Stratagene, La Jolla, CA) as previously described⁶⁷. After harvesting conditioned medium, the viral particles were precipitated using polyethylene glycol 8000 and iodixanol continuous gradient centrifugation as previously described⁶⁸. The genomic titer of purified AAV9 vectors was determined by qPCR that targeted the WPRE sequence.

Measurement of pressure in rat RVLM. Intra-brainstem pressure (IBP) was measured using a

972 blood pressure telemeter by means of the procedure we previously reported^{24,69}. The pressure sensor
973 was placed in rat RVLM using the same approach with the microinjection described above. During
974 IBP measurement, respiration was monitored using a respiration sensor attached to the tested rats.
975 Low-pass (50 Hz) filtered IBP waves were analyzed using LabChart 8 software (ADInstruments).
976 We observed ~0.5-Hz respiration-synchronized IBP changes with ~2.5 mm Hg magnitude (peak to
977 peak) as well as 2-Hz PHM-specific waves with ~1.2 mm Hg magnitude (peak to peak).

978

979 **In vivo analysis of the distribution dynamics of interstitial fluid in rat RVLM using μ CT.**

980 Isovist (Bayer, Berlin, Germany) was stereotaxically microinjected into RVLM of anesthetized 12-
981 week-old male WKY rats following the procedure described above, and visualized using μ CT
982 (inspeXio SMX-100CT, Shimadzu, Kyoto, Japan). After Isovist injection, rats were subjected to
983 two serial brain μ CT scans between which PHM was either applied or left unapplied (kept
984 sedentary) for 30 minutes (Fig. 4e). μ CT images were analyzed using software for 3D morphometry
985 (TRI/3D-BON-FCS64, RATOC System, Tokyo, Japan). Voxels with ≥ 1.02 times signal intensity as
986 compared with that of air was defined as Isovist cluster in rat RVLM.

987

988 **Simulative calculation of the magnitude of FSS on the cells in rat RVLM during PHM.** We

989 calculated interstitial fluid flow-derived FSS imposed on cells in rat RVLM by referring the results

from our μ CT analysis (Fig. 4g,h) to the Henry Darcy's law, which defines the flux density of penetrating fluid per unit time⁷⁰). The velocity of interstitial fluid flow (u) is assumed to approach the superficial velocity (u_∞) and zero ($u=0$) at the cell surface (i.e., a no-slip condition). Using these two boundary conditions together with the Brinkman equation, FSS (τ) at the interstitial cell surface can be obtained as described in Supplementary Table 1⁶⁰.

Cell culture. Primary cultures of astrocytes were prepared from the cortex of neonatal (2–3 days old) astrocyte-GFP mouse³⁷ pups by physical dissociation as previously described⁷¹. Cells were maintained in DMEM/F12 supplemented with 10% FBS (GE Healthcare Life Science, Marlborough, MA), 100 I. U./mL penicillin and 100 μ g/mL streptomycin at 37°C in a humidified incubator (5% CO₂ and 95% air). Culture medium was replaced with fresh one every 3–4 days until confluent. Astrocytes were detached with trypsin/EDTA (0.05% trypsin, 0.53 mM EDTA in PBS), replated at a ratio of 1:10, and grown to approximately 80–90% confluence prior to use.

Mouse neuroblastoma-derived Neuro2A cells (provided from Dr. Yokota, Tokyo Medical and Dental University, Japan), which exhibit neuronal phenotypes and morphology^{39,40}, were cultured in DMEM (Wako, Japan) supplemented with 10% FBS, 100 I. U./mL penicillin and 100 μ g/mL streptomycin at 37°C in a 5% CO₂ incubator.

1008 **Application of FSS to astrocytes or Neuro2A cells in culture.** Astrocytes or Neuro2A cells grown
1009 in a poly-D-lysine-coated 35-mm culture dish (Corning Life Sciences, Corning, NY) were exposed
1010 to pulsatile FSS (average 0.7 Pa) for 30 minutes. As we previously reported^{23,24,35}, a parallel plate
1011 flow-chamber and a roller pump (Masterflex; Cole-Parmer, Vernon Hills, IL) were used to apply
1012 FSS. The flow-chamber, which was composed of a cell culture dish, a polycarbonate I/O unit, and a
1013 silicone gasket, generated a 23-mm-long 10-mm-wide 0.5-mm-high flow channel. To maintain pH
1014 and temperature of culture medium, we used a 5% CO₂-containing reservoir and a temperature-
1015 controlled bath.

1016

1017 **Tissue preparation and immunostaining (immunohistochemical or immunocytochemical**
1018 **analysis).** Rats were anesthetized with intraperitoneal injection of midazolam, butorphanol, and
1019 medetomidine, and perfused transcardially with 4% paraformaldehyde (PFA; TAAB Laboratories
1020 Equipment, Aldermaston, UK). The brainstems were excised and post-fixed with 4% PFA in PBS
1021 overnight at 4°C. The tissues were cryoprotected by soaking in 20% sucrose/PBS for 24 hours and
1022 in 30% sucrose/PBS for additional 24 hours at 4°C. Fixed brainstems were frozen in optimal cutting
1023 temperature (OCT) compound (Sakura Finetek, Tokyo, Japan) and cut into 16 µm-thick coronal
1024 sections using a cryostat (CM3050S; Leica Microsystems, Wetzlar, Germany). Sliced sections were
1025 permeabilized and blocked with 0.1% Tween-20 and 4% donkey serum (Merck Millipore,

1026 Burlington, MA) in Tris-buffered saline, blocked with, and stained by incubating with primary
1027 antibodies at appropriate dilutions followed by their species-matched secondary antibodies. Cell
1028 nuclei were stained with DAPI (Sigma-Aldrich). The slides were mounted with ProLong Gold
1029 Antifade Reagent (Thermo Fisher Scientific) and images were captured with a BZ-9000 digital
1030 microscope system (Keyence, Osaka, Japan).

1031 For immunocytochemistry, cultured cells were fixed with 4% PFA in PBS for 20 minutes at
1032 room temperature (RT) and permeabilized and blocked with 0.1% Triton X-100 and 10% FBS
1033 (Thermo Fisher Scientific) in PBS for 30 minutes at RT. The cells were then incubated with primary
1034 antibodies for 2 hours and then with secondary antibodies for 1 hour at RT.

1035
1036 **Quantitative PCR Analysis (reverse transcription and real-time PCR).** 500 ng of total RNA
1037 extracted from cell culture were subjected to reverse transcription, using ISOGEN II (NIPPON
1038 GENE, Tokyo, Japan) and PrimeScript RT reagent Kit (TaKaRa, Kusatsu, Japan). The resulting
1039 cDNA was subjected to real-time PCR analysis using glyceraldehyde-3-phosphate dehydrogenase
1040 (GAPDH) as an internal control in Applied Biosystems 7500 Real Time PCR System with Power
1041 SYBR Green PCR Master Mix (Thermo Fisher Scientific).

1042 The primers (sense and antisense, respectively) were as follows: mouse *Agtr1a* (AT1R-encoding
1043 gene); 5'-AAAGGCCAAGTCGCACTCAAG-3'

1044 and 5'-TCCACCTCAGAACAAGACGCA-3',
1045 mouse *Gapdh* (GAPDH-encoding gene); 5'-GCAAAGTGGAGATTGTTGCCAT-3' and
1046 5'-CCTTGACTGTGCCGTTGAATTT-3', WPRE (for genomic titration of purified AAV9 vectors);
1047 5'-CTGTTGGGCACTGACAATTC-3' and 5'-GAAGGGACGTAGCAGAAGGA-3'.

1048
1049 **Fluorescent Ang II binding assay.** Six or twenty-four hours after the termination of FSS
1050 application, cultured astrocytes were incubated with Ang II type 2 receptor inhibitor, PD123319
1051 (10^{-6} mol/L in PBS; ab144564, Abcam), for 20 minutes, and then with tetramethylrhodamine
1052 (TAMRA)-labeled Ang II (10^{-8} mol/L in PBS; AS-61181, AnaSpec, Fremont, CA) for 30 minutes.
1053 After 3 times wash with PBS, samples were fixed and subjected to anti-GFP immunostaining to
1054 strengthen the GFP-derived green fluorescence signals and corroborate our analysis on astrocytes
1055 prepared from astrocyte-GFP mice as well as to secure the binding of fluorescent Ang II. Green and
1056 red fluorescence was viewed with a fluorescence microscope (BZ-9000 HS, Keyence). Samples
1057 from astrocytes left unexposed to FSS were prepared and viewed likewise, and provided an
1058 experimental control.

1059
1060 **Hindrance of interstitial fluid movement (flow) by introduction of hydrogel in rat RVLM.** Just
1061 before use, a pre-mixture of polyethylene glycol (PEG) with functional groups (25 g/L in PBS) was

1062 prepared from tetra-armed thiol-terminal (TetraPEG-SH) (Yuka-Sangyo, Tokyo, Japan) and
1063 acrylate-terminal (Tetra-PEG-ACR) (JenKem Technology, TX, USA) PEG solutions as we
1064 previously described²⁴. Tetra-armed polyethylene glycol without functional groups (25 g/L in PBS)
1065 was used as an ungelatable control. For the analysis of hydrogel distribution in rat RVLM, we used
1066 Tetra-PEG-SH fluorescently labeled with a thiol-reactive probe (Merck KGaA, Darmstadt,
1067 Germany). Microinjection of PEG solutions into rat RVLM was conducted as described above.

1068 To specifically analyze the consequences of PHM and hydrogel introduction by minimizing
1069 possible invasive influences of microinjection itself, we gave 1-week recovery time before the first
1070 BP measurement, and then applied PHM to the rats (daily 30 minutes, 14 or 28 days). Immediately
1071 subsequent to post-PHM 24-hour urine collection (Fig. 6a), rats were sacrificed by transcardial
1072 infusion of PFA and subjected to histological analysis.

1073

1074 **Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay.** Rat
1075 RVLM sections were stained using a TUNEL kit (Biotium, Fremont, CA) according to the
1076 manufacturer's protocols, counterstained with DAPI, and then viewed using a 20x objective with a
1077 fluorescence microscope (BZ-9000 HS, Keyence). The nuclei of apoptotic cells were determined by
1078 the existence of green fluorescent patches, and cell apoptosis was quantified by referring their
1079 counts to total numbers of nuclei defined by DAPI staining.

1080

1081 **Measurement of accelerations at human head.** To measure the accelerations at the human head
1082 during treadmill running or VO2R, we fixed an accelerometer (NinjaScan-Light; Switchscience,
1083 Tokyo, Japan) on foreheads with a surgical tape. Vertical acceleration was evaluated using the
1084 software application provided from the manufacture.

1085

1086 **Blood sampling and measurement of parameters in plasma and serum of humans.** Blood
1087 sampling in the human study of protocol 2 was conducted between 12 PM and 3 PM. Participants
1088 were rested in a sitting position for at least 10 minutes before starting the sampling procedures.
1089 After plasma (for catecholamines and renin activity) and serum (for aldosterone and CRP) separation
1090 by centrifugation, we outsourced the measurement of parameters to be tested (BML, Kawagoe, Japan).

1091

1092 **Design and participants of clinical study on antihypertensive effects of vertically oscillating**
1093 **chair riding.** We conducted single-arm clinical studies. The study of protocol 1 (Extended Data
1094 Fig. 5c) was carried out at the affiliated health services facility of Iwai Medical Foundation (Iwai
1095 Keiaien, Tokyo, Japan). The study of protocol 2 (Extended Data Fig. 5d) was carried out at the
1096 National Rehabilitation Center for Persons with Disabilities Hospital.

1097 Subjects were considered eligible if they were 20–75 years old of age and confirmed to have

1098 130–160 mm Hg of SBP at the time of interview for informed consent and eligibility check.

1099 Subjects with mental or psychological illnesses, history or presence of cardiovascular events,

1100 history or presence of severe dysfunction/disorder of liver, kidney, lung, gastrointestinal tract, and

1101 spine, or presence of acute injuries/diseases (e.g., recent traumas and infectious diseases) were

1102 excluded with the exception of those who were given permission for participating in this study from

1103 their primary care physicians. Whereas antihypertensive medication did not disqualify the subjects

1104 (Supplementary Table 2), they were advised not to change their medication from at least one month

1105 prior to the first bout of VOCR through the study period (i.e., up to 8 weeks after the last bout of

1106 VOCR). At a certain (approximately fixed) time point in the morning (typically just before

1107 breakfast), they conducted 3 consecutive measurements of BP (mm Hg) and HR (bpm) using an

1108 automated upper arm-cuff sphygmomanometer, and recorded the values from all those

1109 measurements. These procedures of BP measurement and recording accord with the Japanese

1110 Society of Hypertension Guidelines for the management of hypertension (JSH2019)⁷². Subjects

1111 were directed to start periodical (≥ 3 days per week) BP/HR measurements at least 2 weeks before

1112 the initiation of the intervention (i.e., daily VOCR) and continue to measure BP/HR throughout the

1113 study period using the same sphygmomanometers. Particularly at the study of protocol 2 (i.e., the

1114 study at the National Rehabilitation Center for Persons with Disabilities), participants were advised

1115 to record all the data of those measurements. Those whose BP lowered below the eligibility

1116 requirement of the study (≥ 130 mm Hg of SBP) prior to the initiation of VOCR intervention were
1117 eliminated from the study. Participants were directed to be rested and keep calm for at least 1
1118 minute before starting to measure BP/HR. The mean BP/HR value from 3-time measurements was
1119 defined as “value of the day”, and used for statistical analysis. When BP and HR were measured
1120 and recorded on ≥ 3 days during a particular week in the study of protocol 2, the mean of all the
1121 “value of the day”s through the week was defined as “value of the week”. For the participants who
1122 agreed, periodical BP/HR measurement and recording (≥ 3 days per week) was extended up to 8
1123 weeks after the last bout of VOCR.

1124
1125 **Statistical analysis.** All the quantitative data are presented as mean $\pm$ s.e.m. Parametric statistical
1126 analyses were conducted by paired or unpaired two-tailed Student’s *t*-test for two-group
1127 comparison, and ANOVA with Tukey’s post hoc test for multiple (≥ 3) group comparison, using
1128 Prism software (Version 8, GraphPad Software, San Diego, CA). Differences were considered as
1129 significant at *P* values below 0.05.